

PHOTOSYNTHESIS AND GROWTH OF RICE (*Oryza sativa* L.)
AS INFLUENCED BY POTASSIUM NITRATE AND UREA FERTILIZATION

By

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A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF
THE UNIVERSITY OF FLORIDA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1978

ACKNOWLEDGMENTS

The author expresses her deep gratitude to Dr. Leon A. Garrard, Chairman of the Supervisory Committee, for his inspiration and guidance throughout the period of research.

Deep appreciation is due to Dr. V. E. Green, Jr., Dr. D. E. McCloud, Dr. T. E. Humphreys, and Dr. V. N. Schroder for serving as members of the Supervisory Committee and for reviewing this manuscript.

The author also expresses her thanks to Dr. M. H. Gaskins, Agronomy Department, for providing the greenhouse facilities, and to Dr. N. Gammon and Dr. D. A. Graetz, Soil Science Department, for the use of the Soils Laboratory. Thanks are also expressed to Mr. Bill Spencer, Ms. Mary Rutter, Ms. Jamey Carter, Ms. Becky Witt, and Ms. Margaret Glenn for their assistance during all phases of this research. Special thanks are given Dr. W. T. Haller for supplying chemicals, equipment, and personnel for this research.

The author is thankful to her husband, M. F. Karim, for his understanding, and deep gratitude and respect are extended to her parents for their spiritual support.

Appreciation is given the Agricultural Research Service, U.S.D.A., Southern Region, Stuttgart, Arkansas, for supplying rice seed.

Finally, the author is grateful to the International Rice Research Institute, Philippines, for providing financial support for study.

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Abstract of Dissertation Presented to the Graduate Council
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

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March, 1978

Chairman: Leon A. Garrard
Major Department: Agronomy

In young rice, Oryza sativa L. cv. 'Nato', seedlings, urea enhanced growth and affected carbon/nitrogen metabolism more than nitrate or control (minus N) treatments under both low and high radiation. Efficiency of urea was due to its rapid hydrolysis in shoots and roots, providing reduced nitrogen for amino acid and protein synthesis and additional CO₂ for fixation.

Photosynthesis was highest in 20-day-old seedlings top-dressed with nitrogen. With foliar spray, chlorophyll, total carbohydrate, fresh and dry weights of 20-day-old shoots were highest when plants were subjected to high radiation (1700-1900 μ Einsteins/m²/s). Except for chlorophyll, values for other growth parameters increased by the 30th day. In roots, top-dressing increased total nitrogen content by the 20th day compared to foliar spray, but by the 30th day there was a decrease in total nitrogen content. By

the 30th day, protein content of roots from top-dressed plants had increased, while that of sprayed plants decreased.

Shoots under high radiation showed the highest carbohydrate content in 10-day-old plants when no fertilizer was applied; total nitrogen, protein, and carbohydrate decreased with time in both shoots and roots. Plants subjected to high light and top-dressing showed decreases in photosynthesis, chlorophyll, and protein contents of shoots at 20 and 30 days, with corresponding increases in fresh and dry weights of both shoots and roots. There was an overall decrease in growth from the 20th to 30th day, probably due to depletion of nitrogen.

A comparison of the two light levels showed that shoots and roots had the highest growth rates under high light with either top-dressing or spray application of nitrogen. Rice plants utilized fertilizer more effectively when top-dressed at young stages of growth.

Nitrate reductase activity was highest in shoots subjected to high light and foliar spray. Nitrate-treated shoots always gave higher activities for nitrate reductase. Application of urea also elevated nitrate reductase activity, probably due to an improved nitrogen status in the plant. Nitrate reductase activity was only present in roots of plants under high radiation and was lower in intact tissue of 10-day-old shoots. Low activity in intact tissue assay

may reflect a rate-limiting step in transport of substrate (product) to (from) the site of activity.

Urease activity was highest in shoots and roots of 10-day-old plants, suggesting that the enzyme is important in early development stages. Urea gave a higher response in urease activity than nitrate; top-dressing giving the highest activity. Urease activity in roots at the 20th day was appreciable, being higher with top-dressing. Low light caused no drastic reduction of urease activity in shoots or roots. This was because urease is not light-dependent. Urease activity of 10-day-old intact tissue was lower than that of in vitro assay. Activity of the enzyme was linear over 20-h and highest in tissues of urea-treated plants. Urease activity was low in roots. At the 30th day, urease activity of both shoots and roots was low, probably due to poor plant growth and depletion of nitrogen.

A definite carbon/nitrogen relationship existed in the rice seedlings. Nitrogen fertilization increased protein and carbohydrate contents. Photosynthetic activity also increased with higher leaf nitrogen content. Higher levels of carbohydrate resulted in higher fresh and dry weights of roots and a higher shoot-root ratio. Low light intensity decreased growth by decreasing carbohydrate supply to roots.

These studies suggest that N-Serve, a soil nitrogen stabilizer, had no adverse effect on physiological responses of the plant.

INTRODUCTION

Rice (Oryza sativa L.) is an important agronomic crop that serves as a staple food for more than one-third of the world's population. The crop thrives under a wide range of climatic conditions within an area bordered by latitudes 45° north and 40° south. Rice is usually grown as an "upland" crop or as a "lowland" crop under flooded conditions. It requires abundant water, and the average temperature required throughout the life of the plant ranges from 20 C to 38 C. Rice is generally considered a short-day plant in the sense that short photoperiod decreases its duration of growth. However, there is a wide range of varietal differences ranging from photoperiod insensitive to highly sensitive varieties. The cultivated forms of rice are usually grown as annuals. Depending on the variety, the life cycle ranges from 105 to 150 days from seeding to harvest.

Although rice is the main agricultural crop in most tropical countries and an important crop in the temperate regions, the total yield is low. In the temperate zone countries, where high technology and abundant fertilizer are used, the average yield ranges from 4 to 6 metric tons/ha. In the tropical countries of Asia, the average yield ranges between 1 to 2 metric tons/ha.

The low yield of rice in the tropics is due to a number of factors, such as limited supplies of fertilizer, insufficient water for flooding lowland rice, low light intensity during the monsoon season, as well as unfavorable economic and sociological factors. Although technological developments during the last decade have resulted in the development of non-lodging, nitrogen-responsive, high-yielding varieties of rice, there is still the need for basic research in understanding the various physiological phenomena associated with growth and yield.

The study of the physiology of rice is, without doubt, one of the important areas of research today. It is important to understand the internal processes in order to understand the relationship between the plant and its environment, information necessary for the development of new varieties and new cultural practices. The objective is to maximize the production of dry matter and yield per unit area with minimum energy input.

Photosynthesis and respiration are processes influencing dry matter accumulation (Murata, 1964), and the intensity of solar radiation is a limiting factor in net photosynthesis of plants. In the rice plant, utilization of light is dependent upon the leaf area index (LAI), stature of the plant, and growth response to fertilizer, especially nitrogen. Net photosynthesis is dependent also on the CO_2

concentration of the external air and is influenced by stomates through which CO_2 enters the leaf. Rice is a C_3 plant and CO_2 is lost as a result of photorespiration. C_3 plants do not have the mechanism for trapping CO_2 evolved during the metabolism of photorespiratory substrates, and increasing the pool of CO_2 received by the plant to compensate for the loss due to photorespiration might enhance net carbon fixation. The enrichment of the atmosphere with CO_2 would not be practical on a large scale, and attempts to breed for C_3 plants with anatomical structures and enzymes similar to C_4 plants have not been successful. But, can C_3 plants receive an enrichment of CO_2 through fertilization?

The need for nitrogenous fertilizer for higher growth and increased yield in rice is well established. Although the rice plant can utilize both nitrate and ammonium forms of nitrogen, several investigators have shown the superiority of the ammonium form over nitrate-N. In recent years, urea-N has been found to give an even better plant response than other forms of nitrogen. Although the need and response to nitrogen fertilizers are well known, there is only a limited knowledge concerning the physiology of the rice plants in relation to nitrogen fertilization.

The first step in the utilization of urea is the hydrolysis to NH_3 and CO_2 by the enzyme urease. Webster et al. (1955) and Hinswark et al. (1953) concluded from isotopic

studies that the leaves of a number of higher plants readily hydrolyze urea. Mitsui and Kurihara (1962) showed that CO_2 produced from the hydrolysis of urea is transformed into a dicarboxylic acid. Matsumoto et al. (1966) observed that in rice seedlings urea is metabolised by the control of urease activity in the presence of urea as inducer and that urease activity is suppressed by various proteins or ammonia accumulated during metabolism. Urease activity in extracts of rice seedlings was also observed to be induced after 2 h of incubation. There was also a parallel relationship between enzyme activity and ammonia accumulation. Since urea applied to the plant as a spray is toxic at high levels, most of it must be applied either basally or as top-dressing.

Nitrapyrin (2-chloro-6-(trichloromethyl)-pyridine) has been successfully used with ammonium-N to prevent nitrification. Several investigators (Patrick et al., 1968; Mullison and Norris, 1976) showed that nitrapyrin inhibited nitrification in rice soils by inhibiting the action of Nitrasomonas bacterias. The use of nitrapyrin is also reported to increase the nitrogen uptake, growth, and grain yield in a number of crops (Swezey and Turner, 1962; Patrick et al., 1968; Moore, 1973; Hendrickson et al., 1976). However, the direct effect of nitrapyrin on growth and metabolism has not yet been established with certainty.

This study is concerned with the growth and metabolism of rice plants at the young seedling stage as influenced by nitrogen and nitrapyrin. These include: 1) studying the effect of KNO_3 and urea on young rice plants under two light regimes and two methods of application; 2) verifying previous observations that urea-N promotes greater photosynthesis and growth; 3) demonstrating the presence of urease and identifying the plant sites where urea hydrolysis takes place; 4) studying the properties of nitrate reductase and urease enzymes both in extract and intact tissue of the shoot and root of rice; and 5) determining if the nitrification inhibitor, 2-chloro-6-(trichloro-methyl)-pyridine (N-Serve) has a deleterious effect on growth and metabolism.

CHAPTER I LITERATURE REVIEW

Nitrogen is indispensable to the life and growth of all plants. The atmosphere is the source of elemental nitrogen along with traces of ammonia and other gaseous nitrogenous compounds. The bulk of the nitrogen in plants comes from the soil in the form of nitrate, ammonium ion, and some organic compounds. No matter in what form nitrogen is available to the plant, it must be metabolized to the ammonium form to be utilized in the synthesis of protein and other organic compounds.

The functions of nitrogen in rice plants are as follows: a) increases the chlorophyll content; b) increases the plant height and number of tillers; c) increases protein content of grain; and d) enhances the activity of microorganisms in the soil. The state of nitrogen nutrition can be determined by visual symptoms, by plant analysis at different stages of growth, and by the assessment of dry weight and yield responses. Although the last method is the preferred method, the analysis of different growth components of the plant reflects the amounts and types of nutrients utilized by the plant. The amounts of each nutrient utilized also differ with variety and time, rate, and method of application of nitrogen.

Sources of Nitrogen

The forms of nitrogen supplied to the plant as fertilizer are nitrates, ammonium salts, and ammonium-producing compounds, e.g., urea. Several workers (Hutchinson and Miller, 1909; Arnon, 1937; Arenz, 1941; Kappen and Wienhues, 1942; Burstrom, 1946) reported that cereals (barley, oats, rye, wheat) grew better with nitrates than with ammonia. According to Kellner and Sawano (1884) and Kelley (1911), rice plants grew better with ammonia-N than nitrate-N at the early stages of growth. Shibuya et al. (1938) grew rice plants in nutrient medium and observed better growth with ammonia-N than with nitrate-N. They concluded that the utilization of ammonia and nitrate by paddy rice was affected by the reaction of the medium. De Datta and Magnaye (1969), in a review, stated that in most cases ammonium-containing compounds (ammonium sulfate, ammonium chloride, anhydrous ammonia, etc.) or ammonium-producing compounds (urea) were almost equally effective in terms of yield of grain. Nitrate sources of nitrogen were less effective as preplant fertilizer but may be used as top-dressing at later stages of growth when a surface root system developed (De Datta and Magnaye, 1969). Dijkshoorn and Ismunadji (1972) grew rice plants in flooded soil in pots and observed an increase in growth and nitrogen content in the tops of rice plants when nitrate was replaced by ammonium in the fertilizer.

According to Muhammad and Kumazawa (1974), rice plants absorbed nitrogen both from ammonium and nitrate sources quite rapidly during the early stages of growth, but at the young panicle formation and booting stages the nitrogen from ammonium-supplying sources was translocated more to the youngest growing leaf while the nitrate forms translocated to the newly developed ones.

According to Sorsenbaev and Dobrunov (1976), the presence of ammonium-N in the nutrient solution suppressed the uptake of nitrate-N by roots of a number of rice cultivars. When plants were given ammonium-N alone, there was an increase in synthesis of amides and proteins in roots and shoots: the reverse was observed when only nitrate-N was applied. They also observed that the absorption of ammonium-N was greater than that of nitrate-N until the shooting stage, but nitrate-N was greater thereafter. Marwaha and Juliano (1976) also observed a higher fresh weight, total nitrogen, and soluble protein in young seedlings grown with ammonium-N as compared to those grown with nitrate-N. There was, however, no significant difference in reducing sugar, either in shoot or root, under the two nitrogen regimes. The nitrate-N content of shoot and root was almost 80 and 40 percent higher, respectively, in 10-day-old, nitrate-treated plants than in the ammonium-treated plants.

Methods of Application

Nitrogen is applied either as basal (placement at 10 to 12 cm soil depth), top-dressing (surface application to the water), or as foliar spray. Wada et al. (1973) applied ^{15}N to paddy fields as basal application or top-dressing to check the fate of applied nitrogen. They observed that when nitrogen was applied at young stages, ^{15}N was found in the leaf sheaths and laminae with both application methods, but, at later growth stages, the nitrogen from top-dressing was translocated faster to the leaf blades. Nitrogen top-dressed at maturity resulted in translocation of nearly 70 percent of ^{15}N to the panicles (Wada et al., 1973).

In a farm experiment at IRRI (IRRI Annual Report, 1974), three top-dressings of urea (applied at 100 kg/ha at 10 and 40 days after transplanting and at panicle initiation) gave a slightly lower yield of grain than foliar applications at 15, 28, 41, 51, 58 and 65 days after transplanting.

Taira et al. (1974) observed that top-dressing with nitrogen at the panicle formation stage increased P, and K contents in the ash but decreased ash, P and Mg contents of the dry matter. Top-dressing at heading increased grain protein but reduced dry matter content. Suzuki et al. (1977) top-dressed at $4 \text{ g/m}^2 \text{ N}$ in the rice field at heading stage. They observed increases in glutelin content and total nitrogen contents of brown rice, but application of

nitrogen at an earlier stage had no effect. Yoshida and Padre (1977) observed the recovery of nitrogen from top-dressed material at reproductive stages to be much higher than when it was applied basally.

Singh and Srivastava (1972) applied 30 kg urea-N/ha in trials of wheat, barley, maize, and bajra either in a single top-dressing or as two equal, split dressings and as a single foliar spray. The grain yields of these crops were similar irrespective of the method of application. Jagdish et al. (1972) observed a higher rough rice yield with foliar applications of nitrogen as urea than with soil application. However, in trials of 'Subormati' rice, Helkiah et al. (1973) found no significant difference in the grain or straw yield of 'IR 8' rough rice when urea-N was applied by a number of different soil or foliar application methods. Ramakrishnan (1974) applied different levels of nitrogen by three different methods to 'CO 29' rice at 30 and 45 days after transplanting. He found no significant difference in yield whether the nitrogen was supplied by a single soil application, two foliar applications, or one soil application and one foliar application. This was true for 0, 17, and 34 kg/ha of nitrogen. Paddy yields of 'TN-1' and 'IR 8' rice were found to be greater with 80 kg/ha N applied to the soil in addition to 40 kg/ha N in four foliar sprays than with 100 kg/ha N applied to the soil (Sahu et al., 1975).

A

Response of Nitrogen

The amount of nitrogen assimilated is reflected in fresh and dry weights, nitrogen, chlorophyll, carbohydrate, and protein contents, and photosynthetic activity at different stages of rice plant growth. As early as 1910, Kelley and Thompson determined the total nitrogen and carbohydrate content of various parts of rice plant at harvest. The rice plant root contained 1.55 percent nitrogen and 3.06 percent in the vegetative part. Carbohydrate contents (including reducing sugars, starch, sucrose, pentoses, and some hydrolysable carbohydrates) were 24.1 percent (241 mg/g dry wt) and 26.4 percent (264 mg/g dry wt) in root and shoot, respectively. The soil initially contained 0.25 percent N/g dry wt (2.5 mg/g dry wt). Kapp (1936) analyzed the total nitrogen content of shoot and root at the seedling stage of rice. With 25 ppm N (.025 mg/g fresh wt) as $(\text{NH}_4)_2\text{SO}_4$, he observed a recovery of 3 percent in tops and 2.2 percent in roots.

The percentages of total nitrogen and carbohydrate (total of the non-reducing and reducing sugars and starch) were determined at different growth stages in 'Peta' rice (Tanaka et al., 1964). The percent nitrogen in the plant increased for about 2 weeks after transplanting while there was a slight decrease in carbohydrate content. The percent carbohydrate decreased rapidly until about 4 weeks. The carbohydrate content increased as the nitrogen content

decreased rapidly, indicating a relationship between nitrogen and carbohydrate pools. They observed in 'Peta' that, in early stages of growth, the rate of nitrogen uptake was far greater than the rate of carbohydrate accumulation. Yoshida et al. (1970) observed a close correlation between chlorophyll content and nitrogen nutrition.

Kuriakose and George (1974) applied 30, 60, 90, and 120 kg/ha N on '24-20' rice and analyzed the percentages of sugar and starch accumulated in lamina, leaf sheath, culm, and panicles at tillering, flower initiation, flowering, and maturity. There was a decrease in plant carbohydrate with increasing rates of nitrogen, as observed earlier by Tanaka et al. (1964). The nitrogen content of lamina, leaf sheath, and culm declined from flower initiation onward. However, at flowering, the lamina contained the most nitrogen and the culm the least. At harvest, the panicle contained most of the carbohydrate. The leaf nitrogen content was observed to be 2.58 percent (25.8 mg/g dry wt) at the first joint stage when nitrogen was applied at 90 kg/ha at planting (Wilson and Peterson, 1974). The nitrogen content of the leaf at this stage was positively correlated with grain yield.

Light and Photosynthesis

The rate of photosynthesis in the rice plant is greatly dependent on optimum light conditions, as well as on nitrogen and other nutrients. Net photosynthesis (PN) in rice

increases nearly proportionately with increases in light intensity up to a certain point; however, when the intensity exceeds light saturation points of leaves, the rate of photosynthetic activity may be drastically reduced. At the young stage of the rice plant, low light intensity only slightly affects the dry matter production. But, at later stages, slight cloudiness causes an actual loss of dry matter (DM). Murata and Osada (1959) observed a linear relationship between DM production and photosynthetic activity per unit leaf area at the ripening stage of the grain. Tanaka et al. (1966) observed the relationship between photosynthetic rate and light intensity at different growth stages of 'Peta' plants. They found a curvilinear increase in photosynthetic rate and saturation point with increasing age until panicle initiation, at which stage there was no saturation point. With increased age, there is mutual shading resulting in a decreased net carbon fixation. Stansel (1966) found the period at which light appears to be most critical to be approximately three weeks before to three weeks after the "heading" stage. (Heading is defined as the emergence of the panicle from the flag leaf sheath.)

McDonald (1971) studied the effect of temperature and light on the rate of photosynthesis on 20 varieties of rice. He found 'Bluebelle' to fix $67.4 \text{ mg CO}_2/\text{dm}^2/\text{h}$ and 'IR-8' to fix $42.5 \text{ mg CO}_2/\text{dm}^2/\text{h}$ at 30°C and all light intensities ranging

from 243 to 1313 $\mu\text{Einsteins}/\text{m}^2/\text{s}$. Singh (1973) studied the effect of different light intensities on the vegetative growth of the rice plant, 'Taichung Native-1'. He observed a decrease in plant height but an increase in the number of tillers, number of leaves per plant, leaf area, and dry matter production with increasing light intensity up to 600 $\mu\text{Einsteins}/\text{m}^2/\text{s}$. The increase in growth parameters, except height, was attributed to a higher rate of photosynthesis. Raju and Deb (1975) reduced light intensity to 70, 50 and 10 percent of full sunlight during the vegetative and reproductive stages of two rice cultivars. They observed a decrease in yield in response to lower photosynthesis.

Venkateswarlu (1977) shaded 25-day-old seedlings to 50 to 75 percent of normal incident light and observed a more than 66 percent reduction in DM and yield in 'Sona' rice. He explained that the reduction was due to a decrease in accumulated photosynthetic reserves. Again, Venkateswarlu et al. (1977) supplied low light (40-50 percent of natural light) at different growth phases in rice. They observed a reduction in dry matter content under shading at all stages. Low light intensity appeared to be most critical during ripening phase, resulting in a reduction in yield.

Nitrogen and Photosynthesis

The rate of photosynthesis in the rice plant is dependent to a large extent on the availability of nutrients.

Nitrogen increases the total assimilation per plant by enhancing photosynthetic activity per unit leaf area and promoting expansion of the leaf surface. Mitsui and Nishigaki (1940) observed that nitrogen application increased the photosynthesis per unit of leaf area by about 40 percent over control 10 days after nitrogen application (top-dressing). They explained that nitrogen enhanced the synthesis of enzymatic proteins which participate in the dark reactions of photosynthesis and various other chemical reactions. Nitrogen also participates as a component of chlorophyll in the light reactions. Chlorophyll formation becomes a limiting factor in photosynthesis under low light intensity (Mitsui and Nishigaki, 1940).

Nitrogen is a limiting factor in maximum utilization of light (Tanaka et al., 1966). In 'Peta' rice, they observed that with sufficient light photosynthetic rate increased with increased leaf nitrogen content. Low leaf nitrogen caused the plant to reach light-saturation at a lower light level (50 Klux). High amounts of fertilizer at later growth stages, however, would cause mutual shading due to vigorous plant growth, thereby decreasing photosynthesis.

Ramanujam and Saktharam Rao (1971) observed in 'ADT 27' rice an increase in DM production with an increase in maturity which was positively correlated with photosynthetic rate and level of applied nitrogen.

Kishitami et al. (1972) found that in rice the leaf nitrogen content per unit ground cover was low during early growth, so even leaves with low nitrogen content per unit leaf area were effective in promoting growth. Higher leaf nitrogen per unit ground area promoted growth at later stages.

Norcio and Pantastico (1973), however, did not find any significant difference in the rate of carbon dioxide assimilation with different levels of nitrogen in either 'IR 5' or 'Peta'.

Experiments were conducted by IRRI (IRRI Annual Report, 1973) on photosynthetic rate, specific leaf weight, nitrogen content per unit leaf area, leaf area and stomatal resistance as affected by nitrogen nutrition. It was concluded that photosynthetic rate of young rice leaves is related simply to nitrogen content per unit leaf area and not to specific leaf weight or stomatal activity. Thus, in rice, nitrogen content per unit leaf area is a useful parameter in evaluating photosynthetic potential.

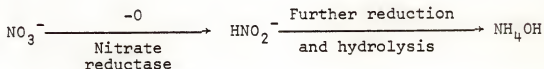
Enzyme Activity

Enzymes are protein catalysts produced by living tissues that regulate the rate of biochemical reactions. In plants, the metabolism of one compound to another requires one or more enzymes. Nitrogen and other nitrogenous compounds available to the plant must be metabolised to the ammonium

form before incorporation into amino acids, proteins or various other nitrogen-containing compounds. Plant enzymes involved in this conversion include nitrate reductase, nitrite reductase, and urease.

Nitrate Reductase Activity

The enzyme nitrate reductase converts nitrate to nitrite. The general scheme for ultimate reduction is



In chlorophyllous tissue, nitrate reductase is found in the chloroplasts.

The activity and presence of the enzyme has been shown by its isolation from many plants. Malavolta (1954) could detect no reduction of nitrate in rice at the seedling stage without special treatment. The presence of nitrate reductase in older rice plants was first reported by Mitsui (1955).

Tang and Wu (1957) showed the adaptive formation of nitrate reductase in 4- to 6-day-old seedlings. They also demonstrated that the enzyme activity is not absolutely absent in ammonia-grown plants. However, nitrate is required

for the formation of nitrate reductase in quantities required by the plant for normal growth (Hageman and Flesher, 1960).

Shen (1969) noted a low nitrate reductase activity in rice seedlings grown in media containing no nitrate and that nitrate reductase activity increased in seedlings with age. As 4-day-old seedlings grown in distilled water had some nitrate reductase activity, the enzyme was thought to be present at low concentrations in rice seedlings as a constitutive enzyme. He observed that NH_4^+ suppressed NO_3^- assimilation but did not prevent the entry of NO_3^- into the cells. He suggested that NH_4^+ inhibits nitrate reductase, the first step of NO_3^- reduction. Marwaha and Juliano (1976) detected a great difference in nitrate reductase activity in rice seedling shoots treated with nitrate-N over that treated with ammonium-N. In roots, they observed only a trace amount of activity in in vitro analysis in both kinds of treatments.

Nitrate reductase activity in plants requires the presence of an electron donor, either NADH or NADPH. Evans and Nason (1953), in their initial work with leaves from young soybeans, believed NADPH to be the preferred cofactor. Beevers et al. (1964) observed that the enzyme from young soybeans was exceptional in that it was capable of directly using either NADH or NADPH as cofactor whereas several other plant species show only traces of activity when NADPH was

added as an electron donor. Kessler and Czygan (1963), Sanderson and Cocking (1964), and Maretzki and Cruz (1967) showed that nitrate reductase from leaves of most plant species had a specific requirement for NADH as the electron donor for nitrate reduction.

Shen (1972) observed that when rice seedlings are treated with nitrate and nitrite, the nitrate reductase formed utilizes NADH and NADPH, with a preference for NADH. When chloramphenicol or a related nitrocompound is the inducer the enzyme formed has a preference for NADPH. Gandhi et al. (1973) observed that nitrate reductase in rice seedlings was specifically activated by preincubation both at 0 and 25 C with low concentration of NADH. They concluded that the nucleotide acted as a positive effector of the enzyme. Later, working with 'IR 8' rice, Shen et al. (1976) showed that nitrate-treated seedlings contained more NADH-nitrate reductase than NADPH-nitrate reductase, whereas chloramphenicol-treated seedlings contained primarily NADPH-nitrate reductase. NADPH-nitrate reductase was shown to utilize NADPH directly as a reductant. Although the enzyme had a preference for NADPH, it reacted as well with NADH.

Light and Nitrate Reductase

A direct relationship between photosynthesis (or some other light-requiring process) and nitrate reduction has been speculated for a long time. Loew (1890c) held that

light affected nitrate reduction indirectly through increased carbohydrate supply and higher respiratory activity. Burstrom (1943) observed that wheat leaves would reduce nitrate in the light but not in the dark. He concluded that this reduction was directly related to a photochemical reaction, or it occurred concomitantly with the fixation and reduction of carbon dioxide. Mendel and Visser (1951) observed in tomato leaf disks a reduction of nitrate in both light and dark, but noted a considerable stimulation by light. The rate of reduction was increased by the supply of glucose or respiratory intermediates. In the dark, reduction was blocked by iodoacetate, an inhibitor of respiration, indicating the need for energy of respiration for reduction (Mendel and Visser, 1951). Candella et al. (1957) observed a great reduction in nitrate reductase activity in cauliflower held in the dark.

Hageman and Flesher (1960), Sanderson and Cocking (1964), and Beevers et al. (1965) observed that the level of nitrate reductase in leaf tissue was influenced by intensity of illumination. However, Beevers et al. (1965) demonstrated that light was not absolutely necessary for induction of nitrate reductase in green leaf tissue as long as sufficient nitrate was present in the induction medium. Spencer (1959) and Filner (1965) indicated that light is not a prerequisite for induction of nitrate reductase in non-green tissue.

Numerous current studies indicate that nitrate reductase activity in plants, including rice, is stimulated by light (Shibata et al., 1969; Travis and Key, 1971; Sawhney and Naik, 1972; Canvin and Atkins, 1974; Naik et al., 1976). Sasakawa and Yamamoto (1977) believed the light effect was due to photosynthesis conducted in green leaves which supply sucrose required for nitrate reductase induction.

Nitrate reduction in roots, though present, is not so pronounced as in shoots. In case of nitrate reduction in root and non-chlorophyllous tissue, Yemm and Willis (1956), observed that the reducing potential was furnished by the respiration of carbohydrates. It was found that the incubation of roots in nitrate or nitrite stimulated carbon dioxide production and increased oxygen consumption to a somewhat lesser degree. Sanderson and Cocking (1964) detected an appreciable nitrate reductase activity in extracts of roots of several plants; however, this activity was consistently lower than that in leaf extracts. Marwaha and Juliano (1976) could find only a trace of nitrate reductase activity in in vitro extracts of roots of rice but an appreciable amount in in vivo analysis.

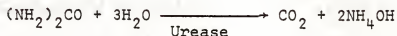
Several authors (Mulder et al., 1959; Randall, 1969) have attempted to compare the in vitro to in vivo nitrate reductase activities. Jaworski (1971) and Streeter and Bosler (1972) observed a higher nitrate reductase activity

in in vivo analysis of soybean leaf tissue. Jaworski suggested the low activity of in vitro assay to be due to both mechanical loss and degradation of the enzyme during extraction. There was, however, a greater reduction in nitrate reductase activity in rice root and shoot treated with ammonium- or nitrate-N with in vivo than with in vitro analysis (Marwaha and Juliano, 1976).

Sawhney and Naik (1972) studied the time-course of activity of nitrate reductase in rice over a 48-h period by in vitro method. They observed that nitrate reductase activity increased up to 12 h in seedlings grown in different light intensities and treated with nitrate-N.

Urease Activity

Urease is a highly efficient, specific enzyme which catalyzes the hydrolysis of urea:



Urease is the first enzyme to be isolated in the crystalline form. Values for the activity of the enzyme from different sources vary widely. Sumner and Hand (1928) obtained 75 units and 15 units of urease per gram of jack bean and soybean meal, respectively. (They defined the unit as the amount of urease which will produce 1 mg of ammonia from urea in 5 min at 20 C.)

Boynton et al. (1953) sprayed urea on 'McIntosh' apple leaves and showed evidence that there was a net gain in protein nitrogen. They also concluded that movement of water-soluble nitrogen from mature sprayed leaves to expanding leaves may be more rapid than to other mature leaves. High concentrations of urea spray resulting in injury to leaves may be due to the accumulation of urea within the leaf rather than to free ammonia.

Kuykendall and Wallace (1954) conducted urea absorption studies in intact but detached leaves of citrus. They observed differences in variety and species and age of leaf in the activity of urease. The extractable urease activity of young leaf tissue in citrus was at least twice that of mature leaf tissue. One half or more of the urea applied was hydrolyzed within 24 h in the intact leaves. They concluded that there was sufficient urease activity in citrus leaf to indicate that urea hydrolysis should never be a limiting factor in the assimilation of foliarly-applied urea.

Cain (1956) studied the primary metabolic products of urea applied to leaves of coffee, cocoa and banana. He found a rapid rate of absorption by all three species. The rate of absorption was more rapid from the lower leaf surfaces of the young leaves. Absorption was complete in coffee and cocoa in less than 24 h and in about 30 h with

banana. Cain also observed a general increase in amino acids in coffee and banana, with a predominance of asparagine. It is, however, not certain whether these amino acids are derived from the hydrolysis of existing plant protein or synthesized in response to urea absorption.

Freiberg and Payne (1957) also studied the foliar absorption of urea and urease activity in banana by use of ^{14}C -labelled urea. They concluded that as much as 65 percent of the applied urea was absorbed within 25 min after application under humid conditions. Absorption was more rapid from the lower surface where there were three to four times more stomata than on the upper surface.

The detection of urea in plant tissue (Takahashi, 1960; Hirose and Goto, 1961) led to the investigation of the pathway of utilization of carbon from ^{14}C -labelled urea by plant roots (Mitsui and Kurihara, 1962). Hirose and Goto (1961) showed that the urease activity was not present or, if present, extremely weak in roots. Mitsui and Kurihara (1962) also observed only weak urease activity, although the rate was increased by the pretreatment with urea. They observed that the carbon dioxide absorbed or produced from urea hydrolysis was incorporated into organic acids in case of excised root but to the sugar fraction in case of intact plants.

Matsumoto et al. (1966) observed that nitrogen metabolism of rice plants is regulated, in part, by the control of urease activity by urea which acts as an enzyme inducer. In addition, ammonia which is a product of urease activity suppressed the induction. Urease activity was induced after 2 h of incubation in urea and then declined due to ammonia accumulation in the extracts during urea hydrolysis. Their results show a very low urease activity in extracts of stem and leaf of rice seedlings.

Nitrification Inhibition

Nitrification is the conversion of the ammonium form of nitrogen to nitrate and nitrite -N in the soil. The bacteria which oxidize NH_4^+ are known as Nitrosomonas spp. Under aerobic conditions the bacteria are most active. Once NH_4^+ is converted to NO_3^- it may be readily leached from the soil, and availability of nitrogen is reduced. A number of chemicals, such as 2-chloro-6-(trichloro-methyl)-pyridine (N-Serve), 2-amino-4-chloro-6-methyl pyrimidine, 2-sulfan-damidotheazole have been found to have the ability to inhibit nitrification of NH_4^+ in soils (Goring, 1962; Patrick et al., 1968; Gasser, 1970; Parr et al., 1971; Prasad et al., 1971; Jaiswal et al., 1972; Kapusta and Varsa, 1972). Of these, N-Serve has been observed to be toxic to Nitrosomonas spp. (Goring, 1962; Norris, 1972; Scott et al., 1975) that causes nitrification of nitrogen, thereby reducing the loss

by leaching. Consequently, there is a greater efficiency of nitrogen fertilizer.

Moore (1973) and Hendrickson et al. (1976) showed an increase in nitrogen uptake and grain yield associated with the use of N-Serve with nitrogen fertilizer. Wells (1977) reported an increased grain yield, plant height and nitrogen uptake in 'Starbonnet' rice by use of N-Serve with urea at the rate of 2.22 kg/ha. Huber et al. (1978) concluded that the use of nitrification inhibitors may markedly increase the efficiency of food production, decrease the incidence of disease, eliminate the need for multiple fertilizer application, reduce energy requirement for growing crops and reduce the pollution potential for nitrogen fertilizer.

CHAPTER II MATERIALS AND METHODS

Rice, Oryza sativa L. cv. 'Nato', seed was obtained from the Agricultural Research Service, U.S. Department of Agriculture, Southern Region, Stuttgart, Arkansas. Plants were grown in a greenhouse under two light intensities at 25 C to 35 C. The quantum flux density in the greenhouse was 1700 to 1900 $\mu\text{Einstein}/\text{m}^2/\text{s}$ (400-700 nm) at mid-day. A portion of the greenhouse was shaded to provide an irradiance of 450 to 600 $\mu\text{Einstein}/\text{m}^2/\text{s}$ (400-700 nm), or about one-quarter full sunlight.

Experiment 1

Plant Material

Seeds were soaked in running water for 2 days to facilitate germination, and the seedlings were then rinsed thoroughly with distilled water and grown in trays of moist vermiculite. The seedlings were grown for 5 days (4 to 5 cm) after which they were transferred to plastic pots containing sand soaked overnight with distilled water. Each pot measured 12 cm in height and 24 cm in diameter and held 60 plants.



Figure 1. 'Nato' rice grown in the greenhouse (30-day-old) under high light regime (L_1) and top-dressed (A_1) with fertilizer.

[Left to Right: 0 (Control), $KNO_3(F_1)$ and Urea (F_2)]

Treatments

Plants were flooded with distilled water for 10 days after which they were placed under different nutrient regimes. Nitrogen source was varied by using either potassium nitrate or urea at the rate of 90 kg/ha N. These materials were added to Hoagland solution which had been prepared without N. Control plants were supplied Hoagland (-N) solution only. Nutrients were applied either as top-dressing or foliar spray. The control and treated pots were kept well-flooded by additions of Hoagland solution (-N) until the 30th day. (Figure 1 shows plants at 30th day.) Samples were taken and analyzed on the 10th, 20th, and 30th day for experiments under high light (L_1) but only on the 30th day for the low light level (L_2).

Fresh samples of shoot and root were pulled carefully, washed thoroughly with distilled water, and analyzed for nitrate reductase activity, total chlorophyll, protein, and urease activity. Another set of treatments with three replicates was used to determine fresh weight of shoot and root, and samples were oven-dried at 60 C for dry weight determination. Dry samples were milled to 40 mesh and used for determining total carbohydrates, NH_4^+ and NO_3^- nitrogen.

Measurement of Photosynthesis

Net photosynthesis (PN) was determined at 20 and 30 days on plants grown under the high radiation regime, but

only at 30 days under the low radiation treatment. PN was determined by measuring the net carbon dioxide uptake by intact leaves in an air-sealed chamber (Wolf et al., 1969).

The carbon dioxide content of air passing over a leaf inside a leaf chamber was measured with a Beckman IR gas analyser, Model 215 B. A photon flux density of approximately $2000 \mu\text{Einstein}/\text{m}^2/\text{s}$ (400-700 nm) was provided by a combination of a single General Electric 400 multivapor mercury (MV 400/HV/E) lamp and a single General Electric Lucalox 4 (LU 400/BU). The leaf chamber was placed beneath the lamps at a distance where the photon flux density, measured by a Lambda Radiometer, Model LI-185, was $1900 \mu\text{Einstein}/\text{m}^2/\text{s}$ (400-700 nm). Therefore, photosynthesis rates were slightly higher than those occurring in the greenhouse. Measurements were made at a leaf temperature of 28 to 30 C and were corrected to standard temperature. Measurements were made on mature leaves at approximately the same positions on the plants. Leaf areas were obtained by measuring the length and width of the leaves and computing from an empirical formula:

$$\text{Leaf area} = \text{Length} \times \text{Width} \times 0.75$$

Chlorophyll Determination

Chlorophyll content was determined according to the method of Arnon (1949); see Figure 2. Absorbance at 645 nm

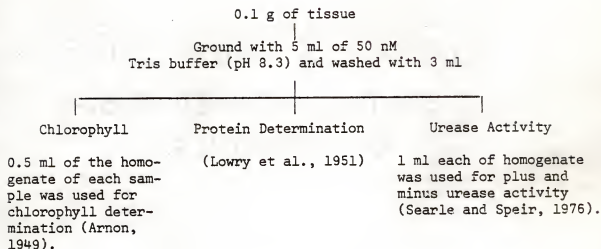


Figure 2. Flow chart of procedure for extracting plant tissue to determine chlorophyll, protein content, and urease activity of 'Nato' rice.

and 663 nm was determined, and chlorophyll content expressed as mg chl/g fresh wt.

Protein

Soluble protein content was determined according to Lowry et al. (1951). One-tenth gram of fresh shoot or root tissue was extracted with 5 ml of 50 mM Tris buffer (pH 8.3) in a glass homogenizer. The homogenate was washed from the homogenizer with 3 ml buffer giving a final volume of 8 ml of extract. A portion of the extract was used for determining chlorophyll content and urease activity. The remaining homogenate was centrifuged at $24,000 \times g$ for 10 min. To an aliquot of 2 ml of supernatant, 2 ml of 20 percent TCA were added and protein was allowed to precipitate for 30 min. This was then centrifuged and the supernatant decanted. The pellet was dissolved in 4 ml of 0.1 N NaOH.

The incubation mixture consisted of 0.5 ml of extracted protein solubilized in 0.1 N NaOH, 5 ml of a mixture containing 100 ml of 4 percent Na_2CO_3 in 0.1 N NaOH, 1 ml of 4 percent (w/v) Na-tartrate and 1 ml of 2 percent (w/v) CuSO_4 . The mixture was incubated at 45 C for 15 min. After incubation, 0.5 ml of 1 N Folic-Ciocalteau phenol reagent was added and mixed immediately. The final volume was made to 10 ml by adding 4 ml of deionized water. The samples then were kept ice-cold until the time of incubation. Absorbancy due to protein was read at 750 nm on a Bausch Lomb Spectronic

70. Protein content was determined from standard curve ranging from 20 to 250 μg BSA/ml and expressed as mg protein/g fresh wt. Triplicate samples were used for protein assay.

Urease Activity

An aliquot of the homogenate in Tris buffer (Figure 2) was used for determining urease activity. The incubation mixture was similar to that of Searle and Speir (1976). It consisted of 1 ml of homogenate, 0.6 ml of 0.5 M Na_2HPO_4 buffer, pH 6.7, 0.4 ml of urea substrate solution (2 mg/ml urea-N) and 0.2 ml of toluene. The mixture was incubated for 24 h at 30 C. After incubation, 8 ml of KCl/phenylmercuric acetate (PMA) solution [150 g KCl dissolved in 900 ml deionized water plus 100 ml of PMA (50 mg PMA/l)] was added to each. To another set of 1 ml of the same supernatant, urea substrate solution was added after incubation and addition of KCl/PMA solution. These were control samples. The vials were recapped and shaken for 1 h. The homogenate was then centrifuged at 5000 x g for 5 min, and the supernatant was assayed for NH_4^+ -N, the enzymatic product, by Nesslerization and the determination of the transmittance resulting from the conversion from urea to NH_4^+ -N was read at 410 nm in a Bausch Lomb Spectronic 70 against the percent transmittance of its own blank. The difference was determined from a standard curve of NH_4SO_4 , ranging from 0 to 20

mg NH_4^+ -N. Urease activity was expressed as $\mu\text{mole/g}$ fresh wt/h. All assays were performed in triplicate.

Nitrate Reductase Activity

Fresh plant tissues (shoot and root) were harvested after 4 to 5 h of illumination and immersed in cold deionized water. The tissues were cut into small pieces, and 0.5 g tissue was weighed carefully and ground with a mortar and pestle (Figure 3). The grinding medium contained 0.1 M Tris (pH 7.3-7.8), 3.0×10^{-4} M EDTA, and 0.01 M cysteine.

The assay mixture consisted of 0.5 ml of 1.36×10^{-3} M NADH, 0.2 ml extract, 1 ml of 0.1 M potassium phosphate buffer (pH 7.5), 0.1 ml of deionized water, and 0.2 ml of 0.1 M KNO_3 . The mixture was incubated at 30 C for 15 min. The reaction was terminated by adding 1 ml of N-1 naphthyl-ethylenediamine·HCl (0.02 percent w/v) and 1 ml of sulfanilamide (1 percent N/V in 1.5 N HCl). Absorbancy was read at 540 nm against its own blank, i.e., complete except for NADH. All assays were performed in triplicate. The nitrate reductase activity was calculated from a standard curve ranging from 20 to 1000 nannomoles KNO_2 with phosphate buffer and KNO_3 but without NADH. The activity of nitrate reductase (NRA) was expressed as $\mu\text{mole KNO}_2/\text{g}$ fresh wt/h.

Total Carbohydrate

The total carbohydrate of dry samples was determined by a modification of the method of Carter et al. (1973).

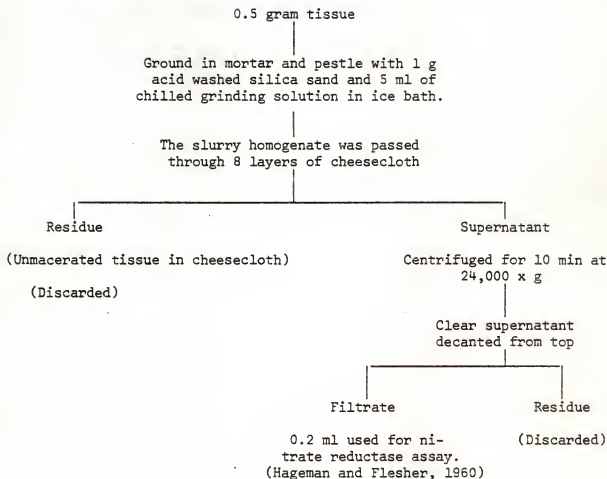


Figure 3. Flow chart of procedure for extracting plant tissue to determine nitrate reductase activity of 'Nato' rice.

A 0.1 g sample was homogenized in a glass homogenizer containing 5 ml of 0.1 M acetate buffer, pH 4.8, and the homogenate was washed with an additional 5 ml of buffer into a 25 ml Erlenmeyer flask. To each flask was added 1.0 ml of a mixture containing 0.8 g amyloglucosidase (from Rhizopus, 2000-4000 units/g, Sigma Chemical Company), 0.02 g taka-diastase (from Aspergillus oryzae, Parke, Davis and Company), and 1.0 ml invertase (liquid concentrate, British Drug House, Ltd.) in 30 ml of 0.03 M acetate buffer, pH 4.8. The flasks were stoppered and placed in a waterbath shaker at 40 C for 3 h, after which the contents were centrifuged at $24,750 \times g$ for 45 min. The supernatant fraction was used for the determination of total carbohydrate as reducing sugar by the Somogyi (1945) modification of the Nelson (1944) procedure.

A 1.0 ml sample of the hydrolyzate was mixed thoroughly in a test tube with 1.0 ml of copper reagent, after which the tube was capped with a glass marble and placed in a waterbath at 100 C for 30 min. The tubes were cooled quickly, 1.0 ml of arsenomolybdate reagent added with thorough mixing, and then brought to a final volume of 5.0 ml. Absorbance of the solution was ascertained using a Klett-Summerson Photoelectric Colorimeter with a No. 54 band filter (500-570 nm). Reducing sugar concentrations were determined from a standard curve utilizing 0 to 0.14 mg/tube

glucose, and total carbohydrate was expressed as mg/g dry wt.

Total Nitrogen

Total nitrogen of the dried ground shoot and root samples were determined by the semi-micro Kjeldahl method (Bremmer, 1965). A 0.1 g sample of tissue was digested with 7 ml of H_2SO_4 -salicylic acid mixture, 1.0 g anhydrous $\text{Na}_2\text{S}_2\text{O}_3$ and 2.0 g catalyst (Kelpak). The digested samples were then distilled with 5 ml of concentrated NaOH (420 g/l). The distillate was received in 15 ml of standard H_3BO_3 indicator up to a volume of about 40 ml. The samples were then titrated to a light purple with standard acid. The sample titre (acid used) was deducted from the blank titre (no tissue). Total N was expressed as mg total N/g dry wt.

Ammonium and Nitrate Nitrogen

Ammonium (NH_4^+ -N) and nitrate (NO_3^- -N) were determined from water extracts (Barker, 1974). A 0.1 g sample of dried ground shoot or root was extracted with 12.5 ml deionized water. The samples were first distilled with MgO into 5 ml of standard H_3BO_3 indicator to remove the NH_4^+ -N. The remaining NO_3^- -N was distilled after reduction to NH_4^+ -N with Devardo's alloy. This fraction was absorbed in another flask containing 5 ml of H_3BO_3 . The distillates were titrated to a light purple color with standard H_2SO_4 . Ammonium and nitrate nitrogen was expressed as mg NH_4^+ -N or NO_3^- -N/g dry wt.

Experiment 2

Time-Course of Nitrate Reductase Activity

Rice plants were grown under greenhouse light condition (1700-1900 μ Einsteins) with basal application (applied as pre-plant with Hoagland solution -N) of fertilizer (90 kg KNO_3 or Urea-N/ha). All other cultural practices were similar to Experiment 1. The shoots and roots were harvested on the 10th and 30th days. The shoot and root parts were separately cut into 5 mm sections, pooled, and weighed into 0.2 g samples. The samples were placed in 25 ml Erlenmeyer flasks. To each flask were added 4 ml of 0.1 M phosphate buffer (pH 7.5), 4 ml of 0.02 M KNO_3 , 4 ml of 5 percent propanol and 4 drops of chloramphenicol (Jaworski, 1971). The flasks were shaken well and vacuum infiltrated for 15 min. Samples were taken at 0 time of incubation, and then the flasks were incubated in a water-bath under laboratory light conditions at 30 C. An identical incubation mixture containing no tissue was used as the blank. Aliquots were taken every 4 h over a 24-h period.

To a 0.4 ml aliquot of incubation mixture were added 0.3 ml of 1 percent sulphanilamide in 3 M HCl and 0.3 ml of 0.02 percent N-1 naphthylethylenediamine·HCl. After 20 min, the assay mixture was diluted with 4 ml of deionized water. All samples were assayed in triplicate. Absorbance was read at 540 nm. Nitrate reductase activity was determined from a

standard curve ranging from 0 to 1000 nannomoles KNO_2 . The activity was expressed as $\mu\text{mole KNO}_2/\text{g fresh wt.}$

Time-Course of Urease Activity

The preparation of plant tissue was similar to that used for nitrate reductase. The incubation mixture consisted of 4 ml of 0.1 M phosphate buffer (pH 7.5), 4 ml of urea substrate solution (2 mg/ml urea-N), 4 ml of 5 percent propanol, and 4 drops of chloramphenicol (0.5 mg/ml). The mixtures were incubated at 30 C in the dark. Aliquots were taken every 4 h over a 24-h period.

The assay mixture consisted of 0.4 ml of incubation mixture, 3.6 ml of 10 percent Na-tartrate and 1 ml of Nessler's reagent. The absorbance due to NH_4^+-N formed was read at 410 nm by a Bausch Lomb Spectronic 70 against its own blank (no urea substrate solution). The urease activity was calculated from a standard graph of 0 to 1000 $\mu\text{mole NH}_4\text{SO}_4$. It was expressed as $\mu\text{mole NH}_4^+-\text{N/g fresh wt.}$

Experiment 3

Treatment with N-Serve

Seeds were prepared in the same way as in Experiment 1. However, the seedlings were planted in pots containing soil. Fertilizer, KNO_3 and urea at the rate of 90 kg/ha were mixed with the nitrification inhibitor, 2-chloro-6-(trichloromethyl) pyridine (N-Serve), at the rate of 2.22 kg/ha. A

set of control pots was treated with fertilizer minus N-Serve. The plants were kept flooded and watered with Hoagland (-N) solution throughout the experiment. Measurement of growth parameters and enzyme assays were performed on the 20th and 30th days. All assay procedures were similar to that of Experiment 1.

CHAPTER III RESULTS AND DISCUSSION

Experiment 1

Effect of Nitrogen Application on Photosynthesis and Growth

The effects of top-dressing (A_1) with nitrate (F_1) and urea (F_2) on the photosynthesis and growth of rice seedlings under a high light intensity regime (L_1) are shown in Table 1 (shoot) and Table 2 (root).

The application of fertilizer as nitrate (F_1) and urea (F_2) increased the chlorophyll content per g fresh weight by the 20th day after addition of nitrogen. Urea application resulted in higher chlorophyll content at both the 20th- and 30th-day plants, the difference between sources being greatest at the 30th day. Yoshida et al. (1970) also observed a close correlation between chlorophyll content and nitrogen nutrition. Application of nitrogen also resulted in a higher rate of photosynthesis ($\text{mg CO}_2/\text{dm}^2/\text{h}$) on both the 20th and 30th days. Although rates were higher on the 20th day, the difference between the treatments was greater at 30th day. The rate of photosynthesis of the treatments at both 20th- and 30th-day observations were 2 to 2.5-fold greater than that of the control (no fertilizer) plants. Differences in photosynthesis among the treatments and between treatments

Table 1. Effect of nitrogen source on net photosynthesis, chlorophyll and total carbohydrate contents, and fresh and dry weights of 'Nato' rice shoots.^a

Nitrogen Source	PN mg CO ₂ dm ² /h	Chl mg/g fresh wt	Total CHO mg/g dry wt	Fresh wt g/seedling	Dry wt g/seedling
<u>10 days</u>					
0	-	2.71	74.68	0.040	0.008
<u>20 days</u>					
0	34.47	3.41	38.84	0.071	0.020
KNO ₃ (F ₁)	73.12	4.06	43.48	0.232	0.038
Urea(F ₂)	78.59	4.43	45.73	0.209	0.037
<u>30 days</u>					
0	22.19	2.30	27.20	0.148	0.026
KNO ₃ (F ₁)	48.75	3.27	43.30	0.721	0.117
Urea(F ₂)	56.60	3.93	51.54	1.114	0.179

^aNitrogen was applied as a top-dressing (A₁) on the 10th day after transplanting. Plants were grown under a high radiation regime (L₁), 1700-1900 μ E/m²/s (400-700 nm).

^bNitrogen source was added to plants with Hoagland's solution (-N) at the rate of 90 kg/ha.

Table 2. Effect of nitrogen source on total carbohydrate, fresh weight, and dry weight of 'Nato' rice roots.^a

Nitrogen source ^b	Total CHO mg/g dry wt	Fresh wt g/seedling	Dry wt g/seedling
<u>10 days</u>			
0	68.78	0.045	0.006
<u>20 days</u>			
0	23.71	0.040	0.006
KNO ₃ (F ₁)	22.38	0.050	0.007
Urea(F ₂)	22.98	0.056	0.007
<u>30 days</u>			
0	26.76	0.046	0.005
KNO ₃ (F ₁)	28.63	0.107	0.009
Urea(F ₂)	35.39	0.254	0.029

^aNitrogen was applied as a top-dressing (A₁) on the 10th day after transplanting. Plants were grown under a high radiation regime (L₁), 1700-1900 μ Einsteins/m²/s (400-700 nm).

^bNitrogen source was added to plants with Hoagland's solution (-N) at the rate of 90 kg/ha.

and control may be a reflection of differences in their chlorophyll contents.

The amount of total non-structural carbohydrate was highest at the 10th day, when the plants had not received any fertilizer. This was probably due to the abundance of soluble carbohydrate remaining after hydrolysis of stored carbohydrate reserve, a condition likely caused by low nitrogen levels. By the 20th day, total carbohydrate of the control was reduced by almost 50 percent. There were, however, somewhat larger amounts in the treatments at both the 20th and 30th days; at the 30th day, the carbohydrate content of F_2 was 20 percent greater than F_1 .

The fresh and dry weights of the seedlings also increased in both F_1 - and F_2 -treated plants. These results agree with the observations of Tanaka et al. (1964) that active absorption and metabolism of nitrogen results in a large increase in dry weight. By the 30th day, the fresh and dry weights of F_2 were approximately 50 percent greater than those of F_1 .

In roots (Table 2), total non-structural carbohydrate content was highest in 10-day-old plants. Carbohydrate content underwent a reduction in all treatments by the 20th day. By the 30th day, the control and F_1 levels of carbohydrate were slightly higher and similar, whereas the F_2 carbohydrate level was about 25 percent higher

than that of F_1 . Although both nitrogen sources promoted root growth as shown by fresh and dry weight values, this was not apparently the cause of carbohydrate level reductions, as the control plants showed a reduction of root carbohydrate without any accompanying growth. It is quite likely that carbohydrate changes could be explained best as the results of utilization of carbohydrate during respiration and carbohydrate translocation to the rapidly growing shoot.

Total nitrogen, ammonium and nitrate nitrogen, and protein of the rice plant shoot and root at 10, 20 and 30th days are shown in Tables 3 and 4. Control plants were comparatively high in total nitrogen and protein on the 10th day or just prior to nitrogen treatment. In the absence of added nitrogen, both total nitrogen and protein decreased with time; by the 30th day, total nitrogen had decreased by 35 percent and protein by 69 percent. With nitrogen additions, there were obvious increases in total nitrogen, NH_4^+ - and NO_3^- -N, and protein content on the 20th and 30th days. As expected, NO_3^- -N was higher in nitrate-treated plants and NH_4^+ -N in urea-treated plants. Marwaha and Juliano (1976) also observed a higher level of NO_3^- -N in rice seedlings treated with nitrate fertilizer. Total nitrogen and protein were both higher in F_2 plant shoots than in F_1 shoots, although this difference was not readily apparent on the 30th day. During the period between the 20th-day sample and

Table 3. Effect of nitrogen source on total nitrogen, ammonium- and nitrate-nitrogen and protein content of 'Nato' rice shoots.^a

Nitrogen source ^b	Total N mg/g dry wt	NH ₄ ⁺ -N mg/g dry wt	NO ₃ ⁻ -N mg/g dry wt	Protein mg/g fresh wt
<u>10 days</u>				
0	15.47	0.34	0.17	32.22
<u>20 days</u>				
0	10.97	0.07	0.13	19.47
KNO ₃ (F ₁)	29.29	0.45	1.76	42.71
Urea(F ₂)	35.03	0.66	0.32	53.17
<u>30 days</u>				
0	10.06	0.09	0.67	9.89
KNO ₃ (F ₁)	32.76	0.86	1.59	35.81
Urea(F ₂)	34.18	0.53	0.27	39.76

^aNitrogen was applied as a top-dressing (A₁) on the 10th day after transplanting. Plants were grown under a high radiation regime (L₁), 1700-1900 μ Einsteins/m²/s (400-700 nm).

^bNitrogen source was added to plants with Hoagland's solution (-N) at the rate of 90 kg/ha.

Table 4. Effect of nitrogen source on total nitrogen, ammonium- and nitrate-nitrogen, and protein content of 'Nato' rice roots.^a

Nitrogen source	Total N mg/g dry wt	NH_4^+ -N mg/g dry wt	NO_3^- -N mg/g dry wt	Protein mg/g fresh wt
<u>10 days</u>				
0	9.24	0.20	0.09	4.10
<u>20 days</u>				
0	6.36	0.05	0.10	3.49
$\text{KNO}_3(\text{F}_1)$	11.46	0.11	0.23	3.16
$\text{Urea}(\text{F}_2)$	11.88	0.66	0.48	5.25
<u>30 days</u>				
0	9.07	0.59	0.29	1.22
$\text{KNO}_3(\text{F}_1)$	14.89	0.45	1.01	3.06
$\text{Urea}(\text{F}_2)$	9.57	0.38	0.22	3.46

^aNitrogen was applied as a top-dressing (A_1) on the 10th day after transplanting. Plants were grown under a high radiation regime (L_1), 1700-1900 $\mu\text{Einstein}/\text{m}^2/\text{s}$ (400-700 nm).

^bNitrogen source was added to plants with Hoagland's solution (-N) at the rate of 90 kg/ha.

the 30th-day samples, the protein content of the seedlings decreased 16 percent in nitrate-treated seedlings and 25 percent in urea-treated plants. These reductions may have been due, in part, to water uptake during elongation and growth.

The roots from control plants (Table 4) showed a steady decline in protein content from the 10th to the 30th day. As in shoots, NO_3^- -N and NH_4^+ -N were higher in the nitrate-treated and ammonium-treated plants, respectively. The application of both nitrate- and ammonium-nitrogen increased total nitrogen in the roots to about the same value in the 20th-day samples; however, nitrate gave a higher value than urea for the 30th-day sample. Both materials appeared to have a sparing effect on the loss of root protein.

The data of Tables 1, 2, 3, and 4 support the contention that urea (F_2) serves as a superior form of fertilizer. Several other investigators have shown that ammonium form of nitrogen was better utilized by rice plants (Shibuya et al., 1938; Dijkshoorn and Ismunadji, 1972).

Effect of Nitrogen Application on the Activities of Nitrate Reductase and Urease

Rice shoots showed significant nitrate reductase activity (NRA) (Table 5). With no fertilizer treatment, activity was very low. Shen (1969) also noted a low NRA in rice seedlings grown in NO_3^- -N-free media but observed an increase

Table 5. Nitrate reductase and urease activity of 'Nato' rice shoots subjected to high light (L_1) and top-dressing (A_1) with nitrate or urea.^a

Nitrogen source	Nitrate reductase $\mu\text{mole KNO}_3/\text{g}$ fresh wt/h	Urease activity $\mu\text{mole NH}_4^+-\text{N/g}$ fresh wt/h
<u>10 days</u>		
0	0.40	1.29
<u>20 days</u>		
0	0	0.37
$\text{KNO}_3(\text{F}_1)$	28.80	0.49
Urea (F_2)	5.05	0.83
<u>30 days</u>		
0	1.02	0.26
$\text{KNO}_3(\text{F}_1)$	161.24	0.41
Urea(F_2)	52.00	0.48

^aNitrogen was applied as a top-dressing (A_1) on the 10th day after transplanting. Plants were grown under a high radiation regime (L_1), 1700-1900 $\mu\text{Einsteins/m}^2/\text{s}$ (400-700 nm).

^bNitrogen source was added to plants with Hoagland's solution (-N) at the rate of 90 kg/ha.

in activity with age of the seedlings. In these studies, nitrate reductase activity increased markedly in shoots of plants supplied with nitrate and to a lesser extent in plants supplied with urea as the nitrogen source. Tang and Wu (1957) and Marwaha and Juliano (1976) also made similar observations that NRA was enhanced by the addition of NO_3^- -N to the nutrient solution. They agreed that NO_3^- -N was not the only elicitor of NRA. Results presented here also agree with Marwaha and Juliano's (1976) observation that NRA in shoots was higher in rice seedlings treated with NO_3^- -N. Shen (1969) believed the low NRA in NH_4^+ -treated plants was due to the inhibition of the enzyme nitrate reductase by NH_4^+ -N. NRA was almost negligible in roots of rice although there was a detectable activity in 10-day-old and 30-day-old roots (Table 6). The results closely concur with Marwaha and Juliano's (1976), who detected only traces of NRA in roots of young rice seedlings.

In contrast to NRA, urease activity (UA) was higher in young shoots of the rice plant (Table 5). As expected, UA in F_2 was greater than that of control and F_1 plants, both in the 20- and 30-day-old shoots. There appears to be a significant UA also in the roots at all three stages, although there was a decline in activity in 30-day-old roots (Table 6). Again, in roots, UA was higher in 10-day-old plants and in those treated with urea as fertilizer.

Table 6: Nitrate reductase and urease activity of 'Nato' rice roots subjected to high light (L_1) and top-dressing (A_1) with nitrate or urea.

Nitrogen source	Nitrate reductase $\mu\text{mole KNO}_2/\text{g fresh wt/h}$	Urease activity $\mu\text{mole NH}_4^+-\text{N/g fresh wt/h}$
<u>10 days</u>		
0	0.27	0.79
<u>20 days</u>		
0	0	0.26
$\text{KNO}_3(\text{F}_1)$	0.10	0.44
Urea(F_2)	0.10	0.63
<u>30 days</u>		
0	0.50	0.17
$\text{KNO}_3(\text{F}_1)$	0	0.06
Urea(F_2)	0	0.08

^a Nitrogen was applied as a top-dressing (A_1) on the 10th day after transplanting. Plants were grown under a high radiation regime (L_1), 1700-1900 $\mu\text{Einsteins/m}^2/\text{s}$ (400-700 nm).

^b Nitrogen source was added to plants with Hoagland's solution (-N) at the rate of 90 kg/ha.

Effect of Nitrogen Applied as Foliar Spray

The effect of foliar application (A_2) of fertilizer on growth is shown in Tables 7 (shoot) and 8 (root). Spray application increased the chlorophyll content of F_1 and F_2 both at the 20th and 30th days compared to controls and to top-dressing (A_1) (cf. Table 1). Also, total chlorophyll content was higher in F_2 than in F_1 at the 30th day. Both nitrogen sources, applied as foliar sprays had little effect on net photosynthesis at the 20th day; however, both sprays more than doubled the net photosynthetic rate by the 30th day. This is in contrast with results from top-dressing experiments where photosynthesis was enhanced by the 20th day (cf. Table 1). Foliar application of the nutrients increased the total carbohydrate contents of the shoots, compared to controls with the 20-day plants having about 30 percent higher values than the 30-day plants. Generally, the total carbohydrate values did not vary greatly from those determined from top-dressed plants (A_1), except that top-dressed plants showed no decline in carbohydrate on the 30th day.

Fresh and dry weights of shoots increased with the addition of nitrogen as a foliar spray (A_2), but not to the extent observed when nitrogen was applied as top-dressing (A_1 , cf. Table 1). In both cases, A_1 and A_2 , it was obvious by the 30th day that urea was the most effective nitrogen source in fresh and dry weight production.

Table 7. Net photosynthesis, chlorophyll and total carbohydrate contents, and fresh and dry weights of 'Nato' rice shoots subjected to high light (L_1) and spray application (A_2) of fertilizer.

Nitrogen source ^a	PN mg ₂ CO ₂ dm ² /h	Chl mg/g fresh wt	Total CHO mg/g dry wt	Fresh wt g/seedling	Dry wt g/seedling
<u>10 days</u>					
0	-	2.71	74.68	0.039	0.008
<u>20 days</u>					
0	34.47	3.41	38.84	0.071	0.020
KNO ₃ (F ₁)	25.87	5.29	46.79	0.339	0.056
Urea(F ₂)	33.84	5.63	50.45	0.341	0.057
<u>30 days</u>					
0	22.19	2.29	27.20	0.148	0.026
KNO ₃ (F ₁)	48.99	3.42	35.03	0.293	0.049
Urea(F ₂)	52.80	5.72	37.66	0.430	0.066

^aNitrogen was applied as a foliar spray with Hoagland's solution (-N) on the 10th day at the rate of 90 kg/ha.

Table 8. Total carbohydrate, fresh and dry weights of 'Nato' rice roots subjected to high light (L_1) and spray application (A_2) of fertilizer.

Nitrogen ^a source	Total CHO mg/g dry wt	Fresh wt g/seedling	Dry wt g/seedling
<u>10 days</u>			
0	68.78	0.045	0.006
<u>20 days</u>			
0	23.71	0.039	0.006
KNO ₃ (F ₁)	10.23	0.044	0.004
Urea(F ₂)	-	0.039	0.005
<u>30 days</u>			
0	26.76	0.046	0.005
KNO ₃ (F ₁)	15.40	0.068	0.006
Urea(F ₂)	14.11	0.094	0.009

^aNitrogen was applied as a foliar spray with Hoagland's solution (-N) on the 10th day at the rate of 90 kg/ha.

Roots of plants receiving foliar sprays showed very poor root growth compared to those which had been top-dressed (cf. fresh and dry weights, Tables 2 and 8), and it was not possible to ascertain the best nitrogen source for root growth in this case. It is possible that this poor root growth was directly related to the very low carbohydrate level in the roots of the treated plants (Table 8). Abundant ammonium nitrogen in the sprayed shoots could act as a sink for available carbohydrate, thus roots would lose carbohydrate to the plant top where it could be metabolized rapidly to amino acids, amines, and protein. This postulate is supported by the data of Table 9.

Table 9 shows the tissue nitrogen status of plants treated with foliar sprays. In general, foliar sprays of nitrate or urea increased markedly the total nitrogen and protein in shoots of treated plants, in much the same manner as top-dressing (A_1) with nitrogen. It is interesting to note that by the 30th day the levels of ammonium-N and protein in the tops of plants sprayed with urea were higher than those found in top-dressed plants. Nitrate sprays also increased ammonium-N in these shoots, but not protein. This appears to support the explanation given above for low root carbohydrate content and poor growth of these roots.

Table 10 gives the nitrogen status of roots from plants which received foliar sprays of nitrate or urea nitrogen.

Table 9. Total nitrogen, ammonium- and nitrate-nitrogen, and protein content of 'Nato' rice shoots subjected to high light (L_1) and spray application (A_2) of fertilizer.

Nitrogen source ^a	Total N mg/g dry wt	NH_4^+ -N mg/g dry wt	NO_3^- -N mg/g dry wt	Protein mg/g fresh wt
<u>10 days</u>				
0	15.47	0.34	0.17	32.22
<u>20 days</u>				
0	10.97	0.07	0.13	19.47
$KNO_3(F_1)$	28.58	0.06	3.22	40.32
Urea(F_2)	32.13	0.13	0.17	49.65
<u>30 days</u>				
0	10.06	0.09	0.67	9.89
$KNO_3(F_1)$	30.11	3.55	0.97	32.02
Urea(F_2)	34.46	4.65	0.36	49.23

^aNitrogen was applied as a foliar spray with Hoagland's solution (-N) on the 10th day at the rate of 90 kg/ha.

Table 10. Total nitrogen, ammonium- and nitrate-nitrogen, and protein content of 'Nato' rice roots subjected to high light (L_1) and spray application (A_2) of fertilizer.

Nitrogen source ^a	Total N mg/g dry wt	NH_4^+ -N mg/g dry wt	NO_3^- -N mg/g dry wt	Protein mg/g fresh wt
<u>10 days</u>				
0	9.24	0.20	0.09	4.10
<u>20 days</u>				
0	6.36	0.05	0.10	3.49
$KNO_3(F_1)$	9.57	0.12	1.45	6.29
Urea(F_2)	8.19	0.41	0.62	7.06
<u>30 days</u>				
0	9.07	0.59	0.29	1.22
$KNO_3(F_1)$	18.71	0.15	0.27	2.43
Urea(F_2)	17.92	1.14	0.71	2.90

^aNitrogen was applied as a foliar spray with Hoagland's solution (-N) on the 10th day at the rate of 90 kg/ha.

While both nitrate and urea increased total nitrogen at 20- and 30-day, there was no difference between the two materials in this regard at either sampling time. Again, nitrate and urea both increased protein levels in roots at both sampling times, with the greater effect occurring on the 20th day. The total nitrogen content went up in roots of 30-day-old plants although the protein content was low. This was probably due to export of protein to the top as insoluble or cell wall protein. As in the case of total nitrogen content, nitrate and urea had about the same effect quantitatively on protein content.

Nitrate Reductase and Urease Activities in Response to Foliar Sprays

Foliar applications of nitrogen greatly increased the nitrate reductase activity of shoots at both the 20th and 30th days as compared to applications by top-dressing. When nitrate was applied as a spray, the nitrate reductase activity at 20 days was 8-fold greater than the activity observed in top-dressed plants and 1.4-fold greater at 30 days (cf. Tables 5 and 11). This increase in enzyme activity was probably the result of rapid nitrate reductase induction by the enzyme's substrate.

It is also interesting that urea application either as top-dressing (Table 5) or foliar spray (Table 11) caused a marked increase in nitrate reductase activity. It is difficult to explain this observation in terms of a specific

Table 11. Nitrate reductase and urease activity of 'Nato' rice shoots subjected to high light (L_1) and spray application (A_2) of fertilizer.

Nitrogen ^a source	Nitrate reductase $\mu\text{mole KNO}_2/\text{g fresh wt/h}$	Urease ^a activity $\mu\text{mole NH}_4\text{-N/g fresh wt h}$
<u>10 days</u>		
0	0.40	1.29
<u>20 days</u>		
0	0	0.37
$\text{KNO}_3(\text{F}_1)$	242.15	0.22
Urea(F_2)	51.04	0.66
<u>30 days</u>		
0	1.02	0.26
$\text{KNO}_3(\text{F}_1)$	231.47	0.35
Urea(F_2)	100.00	0.86

^a Nitrogen was applied as a foliar spray with Hoagland's solution (-N) on the 10th day at the rate of 90 kg/ha.

mechanism; however, an improved nitrogen status of the shoots and increased protein synthesis are probably involved.

Urease activity was highest in 10-day-old shoots (before treatment) and decreased thereafter in control plants (Table 11). As in the case of top-dressing experiments, application of urea by foliar spray resulted in increased urease activity as compared to control plants (cf. Tables 5 and 11). With top-dressed plants, maximum activity was observed in 20-day-old plants, while in sprayed plants maximum activity was found in 30-day-old plants. The generally low levels of urease activity found in treated shoots may indicate that samples were taken at a time too far removed from urea application to show maximum activity. Urea is quickly absorbed by leaf tissue and readily metabolized. Kuykendall and Wallace (1954) observed that urea applied to intact leaves was hydrolyzed within 24 h of application.

Urease activity at later stages may be enhanced by an increased availability of urea to the plant. As rice shoots and roots possess an efficient system of urea hydrolysis, urea hydrolysis in the soil may be prevented by using a specific inhibitor of microorganisms that cause hydrolysis of urea. This would decrease the loss of nitrogen by nitrification and increase the level of urea available to the plant, providing additional pools of CO_2 when hydrolysis occurs in the shoots and roots.

The effects of foliarly applied nitrogen on the activities of nitrate reductase and urease in roots are shown in Table 12. It was interesting to note that while top-dressing nitrate and urea had virtually no effect in inducing nitrate reductase activity (Table 6), the application of nitrate to foliage increased root nitrate reductase activity, particularly in the 20th-day-root samples (Table 12). Nitrate had no effect on urease activity; however, plants sprayed with urea had higher root urease activity on the 30th day.

Effect of Treatment (A_1) and Low Light (L_2) on Photosynthesis and Growth

Low light intensity reduced the chlorophyll of seedlings by 43 percent (Table 13) when compared to seedlings receiving higher radiation levels (cf. Table 1). Nitrogen applications increased chlorophyll contents of shoots at high radiation intensities but had no effect in this regard at lower intensities. In the low radiation intensity regime, nitrogen source appeared to have little, if any, effect on chlorophyll level. Net photosynthesis was also reduced in the low radiation intensity regime by 30 percent in the presence of nitrate (F_1) and 43 percent in the presence of urea (F_2). If these data are adjusted for differences in the rates of photosynthesis of control plants, these reductions become 38 percent and 50 percent for nitrate-treated and urea-treated plants, respectively--differences which may be adequately explained by the differences in chlorophyll content.

Table 12. Nitrate reductase and urease activity of 'Nato' rice roots subjected to high light (L_1) and spray application (A_2) of fertilizer.

Nitrogen source ^a	Nitrate reductase $\mu\text{mole KNO}_2/\text{g fresh wt/h}$	Urease activity $\mu\text{mole NH}_4^+-\text{N/g fresh wt/h}$
<u>10 days</u>		
0	0.27	0.78
<u>20 days</u>		
0	0	0.26
$\text{KNO}_3(\text{F}_1)$	4.54	0.22
Urea(F_2)	0	0.29
<u>30 days</u>		
0	0.50	0.17
$\text{KNO}_3(\text{F}_1)$	1.37	0.19
Urea(F_2)	1.00	0.61

^aNitrogen was applied as a foliar spray with Hoagland's solution (-N) on the 10th day at the rate of 90 kg/ha.

Table 13. Net photosynthesis, chlorophyll and total carbohydrate contents, and fresh and dry weights of 'Nato' rice shoots and roots subjected to low light (L_2) and top-dressing (A_1) of fertilizer.^a

Nitrogen source ^b	PN mg ₂ CO ₂ / dm ² /h ²	Chl mg/g fresh wt	Total CHO mg/g dry wt	Fresh wt g/seedling	Dry wt g/seedling
<u>Shoot (30 days)</u>					
0	26.01	2.12	32.84	0.119	0.023
KNO ₃ (F ₁)	34.17	2.13	24.82	0.114	0.024
Urea(F ₂)	32.28	1.95	25.38	0.138	0.026
<u>Root (30 days)</u>					
0			18.70	0.022	0.003
KNO ₃ (F ₁)	-		9.56	0.018	0.003
Urea(F ₂)	-		3.86	0.009	0.002

^aSeedlings were grown under a photon flux density of 450-600 μ Einsteins/m²/s (400-700 nm). Seedlings were analyzed on the 30th day.

^bNitrogen was applied as a top-dressing with Hoagland's solution (-N) on the 10th day at the rate of 90 kg/ha.

Total carbohydrate contents of both shoots and roots of plants grown in the low light regime were reduced by the applications of either nitrate or urea, with the effect of urea on root carbohydrate being the greatest reduction (Table 13). This was in marked contrast to shoots and roots of plants grown at high light intensity, in which case total carbohydrate in shoots and roots increased with the application of nitrogen (Tables 1 and 2).

Fresh and dry weights of shoots grown under reduced light intensity were increased slightly by the addition of either nitrate or urea. However, this growth response was extremely limited compared to that of plants grown in the high radiation regime (cf. Table 13 and Table 1). This limited shoot growth was apparently at the expense of root growth. Nitrogen fertilization (especially urea) actually reduced fresh and dry weights of roots.

Total nitrogen of the plant shoots grown under reduced radiation increased with nitrogen application, and this increase could not be explained by increases in protein or other measured nitrogenous components (Table 14). As observed previously, urea gave slightly higher values for total N, $\text{NH}_4^+\text{-N}$, and protein than did nitrate (cf. Table 3 and Table 14). While data for roots are incomplete, it may be seen that low light intensity resulted in a drastic loss of protein even in the presence of added nitrate or urea

Table 14. Total nitrogen, ammonium- and nitrate-nitrogen, and protein content of 'Nato' rice shoots and roots subjected to low light (L_2) and top-dressing (A_1) of fertilizer.^a

Nitrogen source ^b	Total N mg/g dry wt	NH_4^+ -N mg/g dry wt	NO_3^- -N mg/g dry wt	Protein mg/g fresh wt
<u>Shoot (30 days)</u>				
0	13.29	0.18	0.09	10.35
$KNO_3(F_1)$	25.58	0.19	4.61	10.91
Urea(F_2)	32.21	0.47	1.80	12.76
<u>Root (30 days)</u>				
0	7.83	0.74	0.22	2.16
$KNO_3(F_1)$	6.93	4.18	0.64	0.76
Urea(F_2)	-	-	-	0.77

^aSeedlings were grown under a photon flux density of 450-600 μ Einsteins/ m^2/s (400-700 nm). Seedlings were analyzed on the 30th day.

^bNitrogen was applied as a top-dressing with Hoagland's solution (-N) on the 10th day at the rate of 90 kg/ha.

(Table 14). Also, the increase in total nitrogen caused by the addition of nitrate under high radiation regime (Table 3) did not occur under the low radiation regime. Tanaka et al. (1964) observed a slightly lower nitrogen absorption in rice subjected to 25 percent full sunlight as opposed to full sunlight. In this case, marked reductions in photosynthesis and the starch accumulation of both root and shoot were also observed.

Reduced Radiation and Enzyme Activity

Nitrogen application to plants grown under the low radiation treatment increased nitrate reductase in shoots about 90-fold and about 20-fold above the control for nitrate and urea, respectively (Table 15). However, neither control nor treatments showed activities as high as shoots of comparably-treated plants receiving the high radiation treatment (Table 5). No nitrate reductase activity was found in the roots of plants grown under low radiation intensities. It is well known that nitrate reductase is induced by nitrate, and that this induction is light dependent, although the mechanism is not well understood (Zucker, 1972). A number of investigators have observed increases in nitrate reductase activity with increases in radiation intensity (e.g. Hageman and Flesher, 1960; Sanderson and Cocking, 1964; Beevers et al., 1965).

Both nitrate and urea enhanced urease activity in shoots of plants subjected to the low radiation intensity treatment, with urea being somewhat more effective in this regard (Table 15). However, shoots from control and treatment plants had less urease activity than shoots from comparable plants under the high radiation regime (cf. Table 5). In contrast to the marked reduction in root urease activity resulting from nitrogen fertilization of plants under high radiation intensity (Table 6), there were increases in urease activity in response to both nitrate and urea in roots of plants from the low light regime. The reason for these increases of the enzyme in roots from the low light intensity treatment is not known.

Effect of Low Light Intensity and Foliar Spray on Rice Seedling Responses

Foliar application of fertilizer under low light resulted in increases in total chlorophyll, carbohydrate, fresh weight, and dry weight of 30-day-old shoots of both nitrogen treatments, as compared both to controls and plants receiving top-dressing under the low light intensity regime (cf. Table 16 and Table 13). Urea, again, appeared to be better than nitrate in eliciting these responses. In contrast to the low light intensity/top-dressing (L_2A_1) experiment where net photosynthesis was stimulated about 31 percent by nitrate and 24 percent by urea (Table 13), neither

Table 15. Nitrate reductase and urease activity of 'Nato' rice shoots and roots subjected to low light (L_2) and top-dressing (A_1) of fertilizer.^a

Nitrogen source ^b	Nitrate reductase $\mu\text{mole KNO}_2/\text{g fresh wt/h}$	Urease activity $\mu\text{mole NH}_4^+-\text{N/g fresh wt/h}$
<u>Shoot (30 days)</u>		
0	0.77	0.16
$\text{KNO}_3(\text{F}_1)$	70.40	0.26
$\text{Urea}(\text{F}_2)$	14.67	0.33
<u>Root (30 days)</u>		
0	0	0.12
$\text{KNO}_3(\text{F}_1)$	0	0.25
$\text{Urea}(\text{F}_2)$	0	0.29

^aSeedlings were grown under a photon flux density of 450-600 $\mu\text{Einsteins}/\text{m}^2/\text{s}$ (400-700 nm). Seedlings were analyzed on the 30th day.

^bNitrogen was applied as a top-dressing with Hoagland's solution (-N) on the 10th day at the rate of 90 kg/ha.

Table 16. Net photosynthesis, chlorophyll and total carbohydrate contents, and fresh and dry weights of 'Nato' rice shoots and roots subjected to low light (L_2) and spray application (A_2) of fertilizer.^a

Nitrogen source ^b	PN mg $\frac{CO_2}{dm^2/h}$	Chl mg/g fresh wt	total CHO mg/g dry wt	Fresh wt g/seedling	Dry wt g/seedling
<u>Shoot (30 days)</u>					
0	26.01	2.14	32.84	0.119	0.023
$KNO_3(F_1)$	15.21	4.09	40.21	0.212	0.044
Urea(F_2)	27.11	3.79	49.29	0.156	0.030
<u>Root (30 days)</u>					
0	-	-	18.70	0.022	0.003
$KNO_3(F_1)$	-	-	6.88	0.020	0.003
Urea(F_2)	-	-	5.64	0.023	0.002

^aSeedlings were grown under a photon flux density of 450-600 μ Einsteins/ m^2/s (400-700 nm). Seedlings were analyzed on the 30th day.

^bNitrogen was applied as a foliar spray with Hoagland's solution (-N) on the 10th day at the rate of 90 kg/ha.

nitrogen source enhanced net photosynthesis when applied as a foliar spray to plants grown under the low light intensity regime (Table 16). In fact, nitrate inhibited net photosynthesis in these plants, perhaps because of an effect on stomatal aperture and gas exchange.

With a few exceptions, the nitrogen status of plants grown under low light intensity with foliarly-applied nitrogen was quite similar to that of plants which were top-dressed with nitrogen (cf. Table 14 and Table 17). There are some differences in tissue levels of nitrate-nitrogen and ammonium-nitrogen; however, these data do not lend themselves to interpretation. A very noticeable difference between the treatments was in the levels of protein in both the shoots and roots. Plants under low light intensity receiving nitrogen by top-dressing had considerable less shoot and root protein than did the shoots and roots of plants that received nitrogen by foliar spray (cf. Table 14 and Table 17). Where protein levels were enhanced, urea appeared to be the most effective nitrogen form in this regard (Table 17). The protein enhancement in this case was probably due to the supplying of abundant ammonium-nitrogen to the leaves which in turn provided acceptor compounds and energy (by photosynthesis) for amino acid and protein synthesis.

Table 17. Total nitrogen, ammonium- and nitrate-nitrogen, and protein content of 'Nato' rice shoots and roots subjected to low light (L_2) and spray application (A_2) of fertilizer.^a

Nitrogen source	Total N mg/g dry wt	NH_4^+ -N mg/g dry wt	NO_3^- -N mg/g dry wt	Protein mg/g fresh wt
<u>Shoot (30 days)</u>				
0	12.07	0.19	0.09	10.35
$\text{KNO}_3(\text{F}_1)$	28.51	0.37	7.82	21.66
Urea(F_2)	29.25	0.22	0.98	31.22
<u>Root (30 days)</u>				
0	7.83	0.74	0.22	2.16
$\text{KNO}_3(\text{F}_1)$	9.53	0.36	0.25	3.46
Urea(F_2)	13.32	0.26	0.69	2.78

^aSeedlings were grown under a photon flux density of 450-600 $\mu\text{Einsteins}/\text{m}^2/\text{s}$ (400-700 nm). Seedlings were analyzed on the 30th day.

^bNitrogen was applied as a foliar spray with Hoagland's solution (-N) on the 10th day at the rate of 90 kg/ha.

Enzyme Activity with Low Light Intensity and Foliar Spray

Nitrate reductase activity was less in low light regime plants which had foliar spray than in those which were top-dressed (Table 18). Again, nitrate was more effective than urea in the induction of the enzyme. Nitrate reductase activity was reduced by about 4-fold in nitrate-treated and 13-fold in urea-treated plants respectively under low light regime (cf. Table 11). No nitrate reductase was found in roots of plants in this treatment.

Urease activity in shoots that received nutrient spray was about the same level as that of leaves from top-dressed plants, and in both cases, urea application resulted in higher urease activity than nitrate application (cf. Table 15 and Table 18). Nitrogen application as a nutrient spray did not increase root urease activity in plants grown under low light intensity (Table 18).

Growth and Carbon-Nitrogen Relationship

Vegetative growth and reproduction in plants may be modified markedly by varying their external and internal conditions. It has been observed that a rather definite carbon to nitrogen relationship influences vegetative growth and reproduction in higher plants. The data presented here strongly emphasize the important relationship between carbon and nitrogen metabolism in plant growth and productivity. This relationship has been the focal point of the many

Table 18. Nitrate reductase and urease activity of 'Nato' rice shoots and roots subjected to low light (L_2) and spray application (A_2) of fertilizer.^a

Nitrogen source	Nitrate reductase $\mu\text{mole KNO}_2/\text{g fresh wt/h}$	Urease activity $\mu\text{mole NH}_4^+-\text{N/g fresh wt/h}$
<u>Shoot (30 days)</u>		
0	0.77	0.16
$\text{KNO}_3(\text{F}_1)$	52.80	0.24
Urea(F_2)	7.00	0.38
<u>Root (30 days)</u>		
0	0	0.12
$\text{KNO}_3(\text{F}_1)$	0	0.10
Urea(F_2)	0	0.13

^aSeedlings were grown under a photon flux density of 450-600 $\mu\text{Einsteins}/\text{m}^2/\text{s}$ (400-700 nm). Seedlings were analyzed on the 30th day.

^bNitrogen was applied as a foliar spray with Hoagland's solution (-N) on the 10th day at the rate of 90 kg/ha.

studies which, indeed, comprise a large portion of the literature in plant physiology.

As early as 1916, Fisher reported that the vegetative growth of a plant was characterized by a low carbohydrate/nitrogen ratio, or a high nitrogen content in proportion to carbohydrate. Kraus and Kraybill (1918) made similar observations and concluded that in this condition there appeared to be an available supply of both carbohydrate and nitrogen, and the balance between them was such as to produce the best vegetative response without leaving a residue of carbohydrate.

According to Hicks (1928), the value of the total-carbon/total-nitrogen ratio varied at different stages of growth for two spring and winter wheat varieties. During the seedling stage, there was a rapid drop in the percentages of both carbon and nitrogen. At this stage, carbon was lost by respiration, and most of the reserve in the endosperm was taken into the embryo. Although no nitrogen was lost, the actual amount being increased by the withdrawal of reserves from the endosperm, the percentage was decreased due to an increase in dry weight of the young plant. In the present study, although there was no large reduction of nitrogen over a 30-day period (cf. Tables 1 and 2), there was an increase in dry weight of shoots. The plants receiving no nitrogen fertilization did show a reduction of total carbohydrate and nitrogen with a corresponding increase in dry

weight of shoot from 10 to 30 days (cf. Tables 1 and 3). Murata (1961) observed that nitrogen fertilization had a marked, promoting influence on protein synthesis but exhibited a complex influence on carbohydrate metabolism; sometimes remarkably promoting the production of carbohydrates, at other times decreasing carbohydrate accumulation. In the present study, protein and carbohydrate contents increased with nitrogen application, particularly with urea.

In rice, several investigators (Fujiwara et al., 1951; Takahashi et al., 1955; Matsushima, 1957) observed an increase in carbohydrate content with lower nitrogen supply at heading. Takahashi et al. (1955) also observed that low nitrogen content resulted in higher starch accumulation in the leaf and stem of rice at the elongation stage. The data of the present study show higher values for carbohydrate than nitrogen (cf. Tables 1 and 3) indicating, perhaps, that the nitrogen level was not high enough to elicit maximum growth.

Several investigators (Mitsui and Ishii, 1938, 1939; Murata, 1965; Osada, 1964, 1966; Osada and Murata, 1965; Murata et al., 1966; Tanaka et al., 1966) concluded that photosynthetic activity measured under normal conditions had a very high, positive correlation with the total nitrogen or protein nitrogen contents of the leaf over a wide range, usually up to 5 percent nitrogen, irrespective of the growth

stage of the rice plant. The present study indicated a higher photosynthetic activity with increasing nitrogen content of the shoot (cf. Tables 1 and 3; also Tables 7 and 10). Too high a nitrogen content, however, results in mutual shading due to vigorous vegetative growth and thereby decreases photosynthetic activity of rice plants (Murata, 1961). Navasero and Tanaka (1966) also noted that rice plants supplied with abundant nitrogen, under a reduced light condition (400 lux) for 13 days, completely lost their photosynthetic activity. Photosynthetic activity was observed to be decreased greatly in plants subjected to one-fourth full sunlight (cf. Tables 1 and 13; also Tables 7 and 16) with either top-dressing or spray application of fertilizer.

In higher plants, chloroplasts have been observed to account for 25 percent of the total dry weight, and 40 percent of the total nitrogen of the leaf (Bonner, 1952). The effect of nitrogen on photosynthetic activity appeared to be related to the production of chlorophyll. Chlorophyll in the rice plant was observed to increase with nitrogen fertilization (cf. Tables 1 and 7) with a corresponding increase in photosynthesis.

An abundant supply of carbohydrate stimulates root development (Dachnowski, 1914; Knudson, 1916; Curtis, 1918). Higher values for fresh and dry weights of roots were

observed with high carbohydrate contents in both shoot and root of rice seedlings (cf. Tables 1 and 2). Harrison (1934) observed in bluegrass that as the supply of nitrogen decreased the relative amount of top growth diminished, while that of the roots and rhizomes increased. Starring (1923) studied the effect of carbohydrate/nitrogen contents on the production of roots on the cuttings of tomato and Tradescantia. He observed that when carbohydrates were absent or low in amount, the cuttings developed no roots. Reid (1926) indicated that a high nitrogen supply plus a readily available supply of carbohydrates appeared to furnish favorable conditions of root growth.

In higher plants, shoots generally grow in the range of the lower carbon/nitrogen ratios, and roots grow at the higher (Hicks, 1928). Reid (1926) found different growth responses in different seedlings, which were related to the chemical composition of the seed. Thus, the higher the nitrogen content in proportion to the carbohydrate content of the seed the higher the shoot/root ratio. In the present study, higher carbohydrate contents resulted in a higher shoot/root ratio (cf. Tables 1 and 2). Low light (cf. Tables 1 and 13) decreased growth of roots by decreasing photosynthate supply to the roots. Brouwer (1962) noted that root growth was not directly related to shading, but it was indirectly related to decreased carbohydrate synthesis

under low light. Low light decreases carbohydrate flow to the roots and decreases root growth and activity (Brouwer, 1966; Brouwer and deWit, 1969). Similar observations are noted in this study with the rice plant (cf. Tables 2 and 13). Low light retarded root growth by decreasing translocation of carbohydrates to the root system. The reduced root development caused a lower absorption of nitrogen resulting in a decreased synthesis of leaf nitrogen, protein, and fresh and dry weights, causing an overall reduction of growth. Hatrick and Bowling (1973) also observed that root metabolism and nutrient uptake were quite dependent on available assimilate and nutrients in the soil. Boote (1976), in a review, concluded that changes in shoot-root ratio result from the interaction of shoot activity and root activity, which are influenced by species differences, age of the plant, aerial environment, defoliation, soil nutrients, and soil environment.

The present study shows differences in carbohydrate level, fresh and dry weights, protein content and other growth factors in response to different nitrogen sources, method of application, and levels of light intensity.

Experiment 2

Enzyme Activity of Rice Seedlings Measured by Intact Tissue Methods

In the intact tissue method of nitrate reductase and urease activity determinations, explants of leaf or root were incubated in an appropriate buffer containing the substrate for the enzyme to be assayed. Enzyme activity was assumed to be directly related to the amount of enzyme product appearing in the solution surrounding the tissue.

Nitrate Reductase Activity

In general, nitrate reductase activity was considerably lower with intact tissue assays than with enzyme extracts of identically-treated tissue (data not shown). On the 10th day, nitrate reductase activity was apparent in both shoots and roots, with the latter tissue having the highest rate. Only traces of the enzyme were found in shoots of 30-day-old plants, and no activity was found in their roots. In 10-day-old shoots, nitrate reductase activities (average rates for 20 h) were 0.026, 0.189, and 0.026 $\mu\text{moles NO}_2^-/\text{g fresh wt/h}$ for control, nitrate-treated, and urea-treated plants, respectively. In 10-day-old roots, the nitrate reductase activities were 0.101, 0.201, and 0.294 $\mu\text{moles NO}_2^-/\text{g fresh wt/h}$ for control, nitrate-treated, and urea-treated plants, respectively (data not shown). It is interesting to note that, in the case of root tissue, preplant nitrate fertilizer

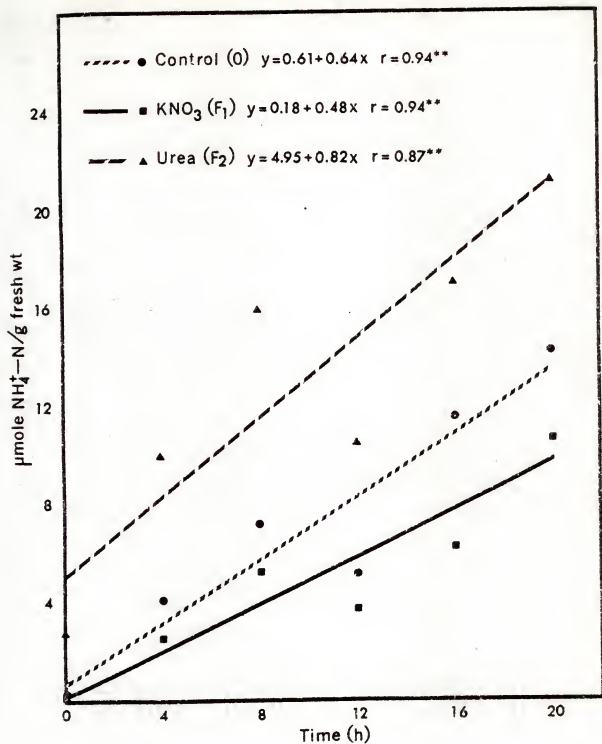
caused a doubling of nitrate reductase activity as compared to the control, while preplant urea application resulted in a 3-fold increase in this enzyme.

It is well known that nitrate reductase synthesis in plants is induced by light and the presence of its substrate, nitrate (Zucker, 1972). Thus, the enhancement of nitrate reductase observed in nitrate-treated plants during this study is easily explained. However, the effect of urea in promoting nitrate reductase activity (even more than nitrate) is somewhat surprising, and any explanation at this time would be a mere conjecture. It may be that urea, because of its rapid mobility in plants, quickly improves the general nitrogen status of the plant, thus making it more responsive in producing nitrate reductase during the 20-h assay period. The generally lower rates of enzyme activity in intact tissue assays may reflect a rate-limiting step during transport of the substrate to the enzyme site, or during the transport of the product back to the bathing medium.

Urease Activity

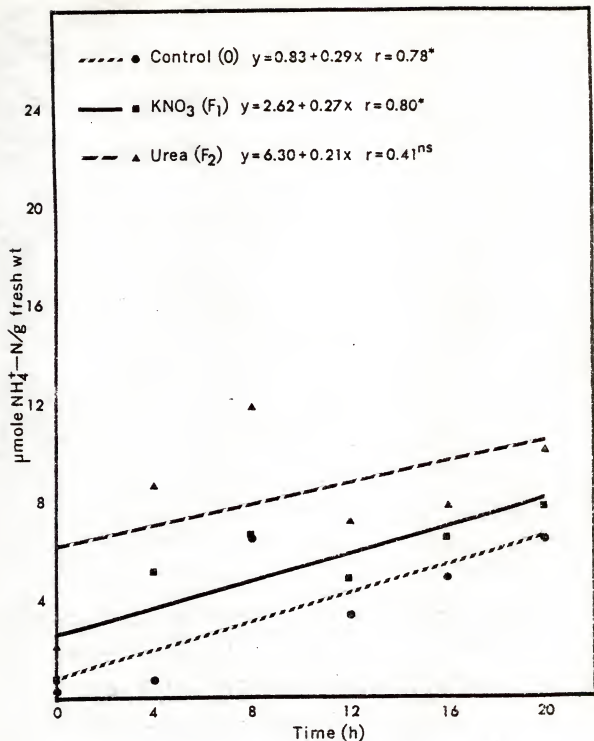
Urease activity of 10-day-old tissues of shoots and roots of 'Nato' rice are shown in Figures 4 and 5. Urease activity of shoots was observed to be linear over a 20-h period in control, nitrate-treated, or urea-treated plants, with r values of 0.94, 0.94, and 0.87, respectively (Figure 4). The values obtained for enzyme activity were lower in

Figure 4: Urease activity of 10-day-old 'Nato' rice shoot
over a 20-h period.
(Standard error of the estimate: Control, 2.03;
 KNO_3 , 1.42; Urea, 3.93.)



**Significant at 0.1 and 0.05 level according to Student's t test.

Figure 5: Urease activity of 10-day-old 'Nato' rice root over a 20-h period.
(Standard error of the estimate: Control, 1.94; KNO_3 , 1.66; Urea, 3.89.)



*Significant at 0.1 level according to student's t test.

^{ns}Non-significant at 0.1 level according to student's t test.

intact tissue analyses than those observed in Experiment 1 where tissue extracts were used in measuring enzyme activity. The rate of activity was observed to be highest in plants treated with urea as preplant fertilizer. Urease activity was about 5- to 7-fold greater in urea-treated plants than in control plants and nitrate-treated plants. The increased rate in urea-treated shoots was probably because rice plants adapt themselves to use urea by preferential induction of urease (Matsumoto et al., 1966).

In the roots (Figure 5), urease activity, although significantly linear for control and nitrate-treated plants, was lower than that of shoots. Again, in the roots, the activity of the enzyme was higher in urea-treated plants. Although urea-treated roots show non-significant r values, the SEE are 3.93 and 3.88 for urea-treated shoot and urea-treated root respectively (c.f. Figure 4 and 5) showing that the deviations of y for fixed x 's for both to be very close. It is interesting, however, to note that unlike nitrate reductase activity, the untreated rice plant root possessed detectable urease activity, perhaps enough to cause considerable hydrolysis of urea and make nitrogen immediately available for amino acid and protein synthesis.

At the 30th day, urease activity of rice shoots and roots dropped drastically (data not shown). The response of the enzyme was not linear over the 20-h period. This was

probably due to poor plant growth as, by the 30th day, the nitrogen source would have been depleted. The observed activity was probably due to the enhancement of urease enzyme formation, when the tissues were supplied with urea substrate solution during the assay.

The use of chloramphenicol, a potent inhibitor of protein synthesis in bacteria, did not inhibit urease synthesis. This indicated that the observed urease activity could be attributed to the rice tissue rather than contaminating microorganisms. Matsumoto et al. (1966) used several inhibitors of protein synthesis in higher plants and concluded that urease synthesis was a de novo synthesis of the enzyme in extracts of rice seedling shoot. Although urease activity is higher in rice shoots, activity of the enzyme was significant and linear also in roots. The presence of urease to hydrolyse urea in both shoot and root enables the plant to better utilize the urea form of nitrogen.

Experiment 3

Effect of N-Serve on the Growth and Metabolism of Rice Seedlings

The effects of soil nitrogen stabilizer, N-serve (nitrpyrin) on the growth and carbon-nitrogen metabolism of 20-day-old 'Nato' rice seedlings are given in Tables 19 through 24. (Data of 30-day-old seedlings not shown.) This chemical inhibits the activity of Nitrosomonas bacteria

which convert $\text{NH}_4^+ - \text{N}$ to $\text{NO}_2^- - \text{N}$ (nitrification) and is used to prevent the loss of nitrogen by leaching of the NO_3^- form (mobile in soil) and denitrification (Patrick et al., 1968; Prasad et al., 1971; Jaiswal et al., 1972). In the present experiment, conditions were created that did not favor the loss of nitrogen by denitrification or leaching, and N-Serve was applied to the soil (washed builder's sand) mixed with preplant fertilizer. This was to determine the effects of the chemical on the plant, per se rather than on soil micro-organisms.

Effect of N-Serve on Photosynthesis, Chlorophyll and Carbohydrate contents and Fresh and Dry Weights

In the absence of nitrogen additions, N-Serve treatment appeared to stimulate photosynthesis of rice shoots (cf. Tables 19 and 20). This stimulation was also reflected in a slightly higher total carbohydrate content and greater fresh and dry weights in N-Serve-treated plants. In the presence of added nitrate, the photosynthetic rate and dry weight/g seedling in shoots of N-Serve-treated plants were also slightly higher than in the absence of the chemical. Urea minus N-Serve treatment resulted in an extremely reduced level of carbohydrate in shoots but increases in both fresh and dry weights (cf. Tables 19 and 20).

N-Serve treatment had a marked effect in increasing root carbohydrate, both in the presence and absence of nitrogen additions (Tables 19 and 20). Also, N-Serve

Table 19. Net photosynthesis, chlorophyll, and total carbohydrate contents, and fresh and dry weights of 'Nato' rice shoots and roots grown in soil treated with N-Serve mixed with fertilizer as preplant.

Nitrogen source	PN mg/ $\frac{1}{2}$ CO ₂ dm ² /h ²	Chl mg/g fresh wt	Total CHO mg/g dry wt	Fresh wt g/seedling	Dry wt g/seedling
<u>Shoots (20 days)</u>					
0	20.95	2.90	48.54	0.337	0.050
KNO ₃ (F ₁)	26.96	3.26	39.78	0.282	0.044
Urea(F ₂)	30.24	3.70	40.38	0.281	0.040
<u>Roots (20 days)</u>					
0	-	-	22.36	0.045	0.005
KNO ₃ (F ₁)	-	-	18.77	0.040	0.005
Urea(F ₂)	-	-	21.20	0.043	0.005

Table 20. Net photosynthesis, chlorophyll, and total carbohydrate contents, and fresh and dry weights of 'Nato' rice shoots and roots grown in soil with no treatment of N-Serve with fertilizer as preplant.

Nitrogen source	PN mg/ ₂ CO ₂ dm ² /h	Chl mg/g fresh wt	Total CHO mg/g dry wt	Fresh wt g/seedling	Dry wt g/seedling
<u>Shoots (20 days)</u>					
0	16.42	3.26	45.89	0.295	0.039
KNO ₃ (F ₁)	22.91	3.42	40.81	0.295	0.040
Urea(F ₂)	31.67	3.86	27.54	0.322	0.047
<u>Root (20 days)</u>					
0	-	-	5.88	0.041	0.005
KNO ₃ (F ₁)	-	-	6.12	0.030	0.003
Urea(F ₂)	-	-	9.18	0.026	0.004

treatment along with nitrate and urea fertilization prevented reductions in root fresh and dry weights which were observed when the nitrogen sources were supplied alone.

Effects of N-Serve on Plant Nitrogen Status

In the absence of nitrogenous fertilizer additions, N-Serve treatment resulted in increases in total nitrogen, NH_4^+ -N and protein nitrogen in shoots of rice plants (cf. Tables 21 and 22). When nitrogen (either nitrate or urea) was applied, NH_4^+ -N was higher in shoots of plants receiving N-Serve than in shoots from plants that were not treated with the chemical. There appeared to be little difference among treatments with regard to total nitrogen contents of the shoots. Nitrate application along with N-Serve increased protein content of shoots compared to that of shoots from the "minus N-Serve" controls. Urea, however, increased the protein content of shoots from plants which had not been treated with the nitrogen stabilizer (Tables 21 and 22).

In roots, N-Serve reduced total nitrogen and protein contents in the absence of applied nitrogen. With nitrate additions, N-Serve increased slightly the protein content of roots and also resulted in higher tissue levels of NH_4^+ -N and NO_3^- -N. With urea addition, total nitrogen was reduced in roots of N-Serve-treated plants (cf. Tables 21 and 22).

Effect of N-Serve on Nitrate Reductase and Urease Activities

In general, the influence of N-Serve treatment on nitrate reductase activities of shoots and roots appeared to

Table 21. Total nitrogen, ammonium- and nitrate-nitrogen, and protein content of 'Nato' rice shoots and roots grown in soil treated with N-Serve mixed with fertilizer as preplant.

Nitrogen source	Total N mg/g dry wt	NH_4^+ -N mg/g dry wt	NO_3^- -N mg/g dry wt	Protein mg/g fresh wt
<u>Shoot (20 days)</u>				
0	24.90	0.52	0.20	28.00
$\text{KNO}_3(\text{F}_1)$	29.61	0.68	1.53	31.06
Urea(F_2)	32.61	0.60	0.34	31.35
<u>Root (20 days)</u>				
0	3.78	-	-	4.31
$\text{KNO}_3(\text{F}_1)$	19.53	0.99	1.71	5.25
Urea(F_2)	8.19	0.47	0.36	5.77

Table 22. Total nitrogen, ammonium- and nitrate-nitrogen, and protein content of 'Nato' rice shoots and roots grown in soil with no treatment of N-Serve with fertilizer as preplant.

Nitrogen source	Total N mg/g dry wt	NH_4^+ -N mg/g dry wt	NO_3^- -N mg/g dry wt	Protein mg/g fresh wt
<u>Shoot (20 days)</u>				
0	19.06	0.34	0.27	26.27
$\text{KNO}_3(\text{F}_1)$	28.32	0.34	1.72	27.81
Urea(F_2)	31.50	0.37	0.46	37.95
<u>Root (20 days)</u>				
0	11.69	0.24	0.21	5.10
$\text{KNO}_3(\text{F}_1)$	17.08	0.15	0.27	5.32
Urea(F_2)	16.67	0.36	0.39	5.77

Table 23. Nitrate reductase and urease activities of 'Nato' rice shoots and roots grown in soil treated with N-Serve along with fertilizer as preplant.

Nitrogen source	Nitrate reductase $\mu\text{mole KNO}_2/\text{mg fresh wt/h}$	Urease activity $\mu\text{mole NH}_4^+-\text{N}/\text{mg fresh wt/h}$
<u>Shoot (20 days)</u>		
0	41.06	0.20
$\text{KNO}_3(\text{F}_1)$	106.00	0.37
$\text{Urea}(\text{F}_2)$	81.06	0.30
<u>Root (20 days)</u>		
0	0	0.13
$\text{KNO}_3(\text{F}_1)$	0	0.52
$\text{Urea}(\text{F}_2)$	0	0.30

Table 24. Nitrate reductase and urease activities of 'Nato' rice shoots and roots grown in soil with no treatment of N-Serve with fertilizer as preplant.

Nitrogen source	Nitrate reductase $\mu\text{mole KNO}_2/\text{mg fresh wt/h}$	Urease activity $\mu\text{mole NH}_4^+-\text{N}/\text{mg fresh wt/h}$
<u>Shoot (20 days)</u>		
0	56.80	0.23
$\text{KNO}_3(\text{F}_1)$	123.20	0.29
Urea(F_2)	82.40	0.41
<u>Root (20 days)</u>		
0	0	0.13
$\text{KNO}_3(\text{F}_1)$	2.90	0.16
Urea(F_2)	0	0.27

be slightly inhibitory, both in the presence and absence of added nitrogen. The exception to this observation was found with shoots treated with N-Serve plus urea (cf. Tables 23 and 24). Here, N-Serve plus urea gave the same rate as urea alone (cf. Tables 23 and 24).

While these data do not clearly suggest an adverse physiological response to N-Serve as it is used under field conditions for nitrogen stabilization, they do suggest that the material per se is biologically active in higher plants and raise the possibility of a masked stress factor during crop production.

SUMMARY AND CONCLUSIONS

1. Effects of light and nitrogen application were apparent in 'Nato' rice seedlings. Photosynthesis was higher in 20-day-old seedlings with top-dressing compared to foliar application of fertilizer. At the 30th day, photosynthesis was about the same in both methods of application under high radiation.

2. On the 20th day, chlorophyll, total carbohydrate, fresh and dry weights of shoots were lower with top-dressing than with foliar spray. Except for chlorophyll content, which remained the same, other growth parameters showed the reverse situation in 30-day-old plant shoots. There was no difference in total nitrogen and protein contents of 20- and 30-day-old shoots under high light with either top-dressing or foliar spray. Total carbohydrate and fresh and dry weights were higher in 20- and 30-day-old roots when top-dressed plants were subjected to high light. Total nitrogen content of roots at the 20th day also remained high with top-dressing but decreased at the 30th day. In roots, protein content varied inversely with total nitrogen. Foliar spray increased the total nitrogen content at the 30th day. Protein content of roots increased with top-dressing but decreased with foliar spray in 30-day-old roots.

3. Shoots under high radiation showed comparatively high levels of total nitrogen and protein on the 10th day or just prior to nitrogen treatment. Total carbohydrate content was highest in 10-day-old plants. In the absence of added nitrogen, total nitrogen, protein and carbohydrate decreased with time in both roots and shoots. Plants subjected to high light and top-dressed showed decreases in photosynthesis, chlorophyll, and protein in shoots at 20 and 30 days. There was an increase in fresh and dry weights of both shoots and roots from the 20th to the 30th day. Carbohydrate and total nitrogen contents remained more or less the same in shoots of 20- and 30-day-old plants. In roots, only carbohydrate content showed an increase from the 20th to the 30th day. Protein content decreased in both shoots and roots during this period, although the total nitrogen content remained the same. The overall decrease of growth from the 20th to the 30th day was probably due to depletion of nitrogen.

4. Plants subjected to high light and top-dressing showed increased growth by all parameters at the 30th day compared to the low radiation regime. With spray application and high radiation, photosynthesis, fresh and dry weights, total nitrogen, and protein contents were higher in 30-day-old shoots than in shoots that were grown under low light.

Spray application caused chlorophyll contents to be the same in both light regimes. In roots, with the exception of protein, all growth parameters were higher in high radiation and with fertilizer applied as foliar spray.

5. High light intensity caused an overall greater growth with either top-dressing or spray application of fertilizer than low light intensity. This is due to higher rates of photosynthesis and nutrient uptake.

6. Under low light intensity, photosynthesis of rice was greater with top-dressing of fertilizer than with foliar application. Chlorophyll, carbohydrate, fresh and dry weights, and protein contents were higher in 30-day-old shoots given foliar spray. In roots, although fresh weight, total nitrogen, and protein contents were higher with foliar spray, dry weight was approximately the same with both methods of application.

7. Nitrate reductase activity was highest in both 20- and 30-day-old shoots subjected to high light and nitrate fertilization compared to control (minus nitrogen) or urea fertilization. This was also the case under low radiation treatments. The present observations also show that foliar spray resulted in 8-fold increase of nitrate reductase

activity at the 20th day and 1.4-fold at the 30th day compared to top-dressed plants. This increase in enzyme activity was probably the result of rapid nitrate reductase induction by the enzyme's substrate. Urea application, either as top-dressing or foliar spray, also increased nitrate reductase activity--probably due to an improved nitrogen status of the plant. The activity of the enzyme was low in roots under high radiation and absent when plants were subjected to low radiation. Nitrate reductase activity, though very low, was detectable in both shoot and root of 10-day-old seedlings kept under high light conditions.

8. Urease activity was higher in shoots and roots of plants fertilized with urea as both top-dressing and spray application compared to control and nitrate treatments, with top-dressing giving the highest activity values. In roots, urease activity was appreciable--the activity being higher with top-dressing than with foliar spray at the 20th day. Urease activity was highest in 10-day-old shoots and roots when the plants had not yet received any fertilizer. The enzyme appears to be very active at this stage of growth.

9. Reduction of urease activity in shoots and roots subjected to low light was not as drastic as with nitrate reductase activity. This is probably because urease activity

is not light dependent. The reduction observed in urease activity may result from decreased substrate absorption and transport along with other reduced metabolic processes which characterized an "energy poor" condition. With top-dressing and high light intensity, urease activity in shoots and roots decreased during 20 to 30 days compared to spray application where both shoots and roots of urea-treated plants showed an increase in activity with time.

10. This study clearly shows the superiority of urea (compared to nitrate) as a source of nitrogen for rice seedling growth. The higher rate of growth with urea was probably due to its rapid hydrolysis in both shoots and roots, quickly providing reduced nitrogen to be used in amino acid and protein synthesis. The hydrolysis of urea also provides additional pools of CO_2 for fixation in plants.

11. A definite carbon/nitrogen relationship existed in the rice plant. Nitrogen fertilization increased protein and carbohydrate contents. The plants showed higher values for carbohydrate than for nitrogen, indicating that nitrogen content was not high enough to elicit maximum growth. Photosynthetic also increased with higher leaf nitrogen content. Higher fresh and dry weights of roots were observed with higher carbohydrate contents of both shoot and root.

Higher carbohydrate content resulted in a higher shoot-root ratio. Low light intensity decreased growth of root by decreasing carbohydrate supply to the roots.

12. Nitrate reductase activity of 10-day-old plants with preplant fertilizer was considerably lower with intact tissue assays. Both nitrate and urea promoted nitrate reductase activity. The low activity of enzyme in intact tissue assays may reflect a rate-limiting step in transport of the substrate to the site of activity and the transport of the product of reaction to the incubation medium.

13. Urease activity of 10-day-old intact shoots and roots was lower than in in vitro assay. Activity of the enzyme in shoots was linear over a 20-h period in control, nitrate-treated or urea-treated plants. In roots, urease activity was lower than that of shoots. In both shoots and roots, activity of the enzyme was higher in urea-treated plants. Urease activity of roots and shoots was low at the 30th day. This was probably due to poor plant growth and depletion of nitrogen.

14. Studies of N-Serve, a nitrogen stabilizer, did not suggest any adverse physiological response but suggested that the material per se is biologically active in higher

plants and raised the possibility of a masked stress factor during crop production.

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BIOGRAPHICAL SKETCH

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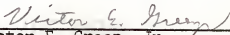
Nilufer is married to M. F. Karim.

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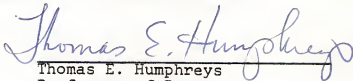
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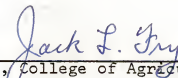
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